ECOLOGICAL GENETICS OF *XANTHIUM*

by

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This thesis contains no material which has been accepted for the award of any other degree or diploma in any University and, to the best of my knowledge and belief, it contains no material previously published or written or the result of work by another person, except when due reference is made in the text.

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SUMMARY

The two species of the genus *Xanthium* are very successful colonizers of fluctuating environments in Australia. The genetic structure of populations and the strategies of colonization, of the two species were analysed to determine what factors have been responsible for their success in these environments.

X. spinosum and 4 races of *X. strumarium* were introduced separately and in different parts of Australia. The distributions of the two species overlap but *X. spinosum* has a more temperate range than *X. strumarium*. *X. chinense* is sympatric with the other 3 races of *X. strumarium* in different parts of its range, but the geographical ranges of the other 3 races are distinct from each other. *X. strumarium* predominately occurs along river banks and associated flood plains, whereas *X. spinosum* is a common weed of disused cultivations and disturbed areas.

Plant populations, as for other organisms, have been found to have considerable amounts of allozyme variation. Analyses of *Xanthium* populations for allozyme variation showed that *X. spinosum* and 3 of the races of *X. strumarium* were monomorphic at all loci studied. Within races there was fixation of alleles at loci but at some loci, races were fixed for different alleles. With the exception of *X. italicum*, the only allozyme variation present was between races and species. This genetic uniformity in qualitative variation was compatible with selective neutrality on the basis of the neutral charge state model.

All races and species of *Xanthium* exhibited considerable phenotypic variation in natural populations. *X. italicum* had the highest total phenotypic variation in the field. The genetic and environmental components of the quantitative variation were measured. All species had considerable phenotypic plasticity, but the races of *X. strumarium*

and *X. spinosum* nearly all had significantly different levels of plasticity from one another. The two most successful colonizers namely *X. spinosum* and *X. chinense* appeared to have different genetic strategies of colonization. *X. chinense* not only had high plasticity but as well it showed significantly lower quantitative genetic variation than the other 3 races and these two factors combined to make *X. chinense* the most successful colonizing species of *Xanthium* in Australia.

There was little evidence of genetic adaptation by the races of *X. strumarium* since their introduction, and the different strategies employed by the races of *X. strumarium* were most likely developed in their native habitats in response to strong selection pressure. Thus the races of *X. strumarium* were primarily preadapted to the ranges they successfully colonized in Australia. Both species have considerable plasticity in germination, and dispersal processes and these contribute considerably to the success of these species as colonizers of Australian environments.

CHAPTER 1

INTRODUCTION

In the past 9 years a vast amount of evidence has accumulated attesting to the ubiquity of electrophoretically detectable genetic variation in natural populations of plants and animals. The average genic heterozygozity appears to be about 6% in vertebrates and 15% in invertebrates [Selander and Kaufman, 1973b; Lewontin, 1974; Nei, 1975]. The question of whether the majority of these observed protein polymorphisms arise as a result of selection [Clarke, 1970; Ayala *et al*, 1972; Johnson, 1973] or through purely random processes acting on selectively equivalent types [Kimura and Ohta, 1971] has stimulated numerous theoretical and experimental studies in the field of evolutionary genetics. At this point in time, it is still largely unresolved as to what proportion, if any, of the electrophoretic variation observed in natural populations is adaptively neutral.

The neo-classical school [see Lewontin, 1974] claims that almost all the observed variants are due to the passage in and out of populations of 2 classes of mutations. One class is selectively neutral while the other class is under selection, but these alleles are deleterious and are being eliminated from the population. The neo-classical view also allows occasional heterotic mutants but these are rare and only form a very small proportion of the genome. On the other hand, the balanced view states that the genetic variation is preserved by natural selection and is significant for adaptive

evolution. Direct evidence for the action of natural selection in the maintenance of polymorphism, although often claimed, has very rarely been demonstrated [Lewontin, 1974; Nei, 1975].

1.1. Neutral Charge State Model

Until very recently discussions relating to selective neutrality of protein polymorphisms have been based on the infinite alleles model of Kimura and Crow [1964]. This model assumes that whenever a mutation occurs it represents an entirely new allele and that furthermore, all protein variants are detectable. However, electrophoretic techniques detect only a fraction of the genetic differences for proteins within populations [Lewontin and Hubby, 1966; Bernstein *et al*, 1973; Singh *et al*, 1974] and hence this model is inappropriate to apply to electrophoretically determined polymorphisms. Marshall and Brown [1975] calculated that at least 74% of mutations are electrophoretically silent.

As a consequence, models have been developed recently which are based on mutational events that are detectable electrophoretically [Ohta and Kimura, 1973, 1974; King and Ohta, 1975; Marshall and Brown, 1975]. These more realistic models should generate qualitative and quantitative predictions directly applicable to electrophoretic data. The neutral charge state model is based on the assumption that each electrophoretically detected mutant is not unique in character but a result of a step-wise recurrent event. The charge state model assumes that mutation occurs with frequency μ to novel alleles, but of these a proportion α is not distinguishable electrophoretically from the parent allele, while a proportion β gives rise to a protein differing

by 1 unit of electric charge from the parental protein and a fraction γ differs by 2 units of charge. Ohta and Kimura [1973] showed that the effective number (n_e) of electrophoretically detectable, selectively neutral alleles in a finite population is given by $n_e = \sqrt{(1 + 8N_e\mu\alpha)}$ for $\gamma = 0$ where N_e is the effective population size and μ the mutation rate. The step-wise changes in charge state lead to a number of distinct classes of electrophoretic variants. These charge classes are genetically heterogeneous and consist of a number of electrophoretically silent alleles. In contrast to the infinite allele model, the common alleles are in the middle of the sequence on electrophoretic gels [Brown *et al*, 1975]. The ratio of the effective number of the actual number of alleles ($\frac{n_e}{n_a}$) is larger and hence allelic frequencies tend to be more evenly distributed in this model, and thirdly the variation of allele number is less in the charge model than in the infinite allele model. In other words, gene frequencies are more uniform for this model compared to allelic frequencies in the infinite alleles model.

The observed protein polymorphisms of *Drosophila* were tested for fit to the theory of neutral protein variation on the basis of the charge state model [Ohta, 1975; Latter, 1975]. The observed and theoretical distributions agreed quite well, except that there was an excess of rare alleles in the observed distributions. This excess is removed if all alleles are slightly disadvantageous [see also King and Ohta, 1975]. Thus the gene frequency data for enzymes in *Drosophila* can be accounted for by mutation-selection balance at nearly all loci and the selection against mutants is very low with NS values in the range 1-3. Another possible explanation of the excess or rare alleles is that the observed polymorphisms are not in equilibrium.

Use of the charge state model as a basis to test whether experimental data agree with the neutral mutation theory is hampered by the fact that, in contrast to the infinite allele model, the theoretical equilibrium gene frequency distribution is not known for the neutral charge class model. Perhaps with further development of the mathematical theory of the charge state model such tests as Ewen's [1972] may be validly applied to test whether observed protein polymorphisms are selectively neutral or not. Ewens and Feldman [1975] are of the opinion that "the gap between the real models probably satisfied by the data, and those so far used, is too large to validate the testing procedures" now available.

In the light of this, and accepting the charge state model as the most realistic available for electrophoretic data at the present time, is there a fruitful experimental electrophoretic approach available, which can contribute to the resolution of the conflict between selective neutrality and balance theories of protein polymorphisms? I believe there is.

Although it is acknowledged that it is virtually impossible to accurately quantify such parameters as N, s, μ and m (population size, selection coefficients, mutation rates and migration rates respectively) for natural populations, it would seem that for certain organisms qualitative statements could be made about some of these parameters which would lead to predictions on the basis of neutral mutation theory, about the nature of the genetic structure of populations of these organisms. For instance, in terms of the charge state model the differentiation of small isolated frog, mouse and human sub-populations could be the result of random drift [King and Ohta, 1975; Lewontin, 1974]. If selective neutrality is the main force maintaining

genetic polymorphisms, then it could be predicted that species occupying very fluctuating environments and going through extreme "bottlenecks" fairly regularly would exhibit considerable inter-population differentiation with respect to enzyme polymorphisms, as a result of random drift. The extent of the interpopulation differentiation will also be influenced by the amount of migration (or gene flow) between populations.

Plants are the obvious organisms for which the relative importance of these population parameters can at least be quantitatively assessed. As Lewontin [1974] so aptly wrote - "with their immense variety of breeding systems plants will be extremely important for comparative studies and for sorting out the forces influencing allozyme variation." Annual plants, especially inbreeders, are very amenable to study in the field situation and to manipulate in experimental studies in comparison to most animal species.

1.2. Genetic Variation in Self-fertilizing Annuals

For annual plants the fluctuations in size and occurrence of populations can be estimated along with the relative importance of environmental and ecological factors in determining these fluctuations. Until very recently there have been very few studies of allozyme variation in plants, with the most detailed being those on colonizing inbreeding annuals. As colonizers, details of the history of their introductions are often known, and although such aspects of the problem are often ignored, many of the paradoxes of genetic variation "may stem entirely from the application of equilibrium explanation to historical phenomena" [Lewontin, 1974]. The genetic structure of populations of several inbreeding species have been analysed in some detail.

An early viewpoint considered that populations of inbreeding plants were genetically uniform [Stebbins, 1957]. Studies carried out in the last 10 years however, have shown that the amount of genetic variability for both qualitative and quantitative characters in populations of inbreeders is of the same order of magnitude as for outbreeding species [Allard *et al*, 1968; Allard and Kahler, 1971]. Self-fertilizing plants are often more variable genetically between families for quantitative characters whereas outbreeders are genetically more variable within families. Environmentally-induced variation (or plasticity) is common in annual plants [Bradshaw, 1965] but nevertheless there are normally many different genotypes in populations of inbreeding annuals [Iman and Allard, 1965; Kannenberg and Allard, 1967; Allard *et al*, 1966]. The colonizing species that have been analysed for allozyme variation are mainly inbreeding grasses, which form fairly stable populations. These have been found to contain quite substantial levels of genetic variation [Marshall and Allard, 1970a; Allard and Kahler, 1971; Clegg and Allard, 1972; Brown *et al*, 1974]. For inbreeding species, in contrast to outbreeding organisms, the level of heterozygosity decreases as the amount of self-fertilization increases [Allard and Kahler, 1971]. In practice this means that large populations of inbreeders can be analysed for allozyme variation comparatively easily. The effective size of a highly inbreeding population of size N is N/2, and in these populations, as a result of the low outcrossing, the amount of recombination will be very small. Such situations could be expected to lead to linkage disequilibrium. A population is in linkage equilibrium if the differences between the products of frequencies in coupling phase and those in repulsion phase are zero.

In fact, the level of inbreeding has a similar effect on linkage disequilibrium as does tightening of linkage [Allard *et al*, 1968]. Strong linkage disequilibria has been observed in the predominately selfing plants, barley and *Avena barbata* [Clegg *et al*, 1972; Allard *et al*, 1972; Hamrick and Allard, 1972].

There have been no extensive genetic studies of inbreeding annuals in highly fluctuating environments. Species in such situations are very often colonizing species, and moreover are usually r-strategists rather than k-strategists [MacArthur and Wilson, 1967; Pianka, 1970]. They are "continually" colonizing their habitats and, in fact, can often partly depend on changes in the environment for successful re-establishment each year. Under extreme environmental conditions the reduction in population size for such species may become so large that random drift becomes an important force in determining the patterns of allozyme variation.

1.3. The Strategies of Colonizing Plants

Plant species, which had spread from their indigenous habitats to become common in a diversity of habitats, most of which became available as a result of man's activities, are considered to be successful colonizing species. Colonizers can be divided into 2 main types (i) those that are preadapted for the environments they are introduced into and (ii) those that require adaptation to their new environments before becoming successfully established.

There is considerable variation in the introduced species *Trifolium subterraneum* in Australia, but this is considered to be as a result of many introductions from a wide range of native environments

rather than because of any significant genetic adaptation since its introduction [Morley, 1960; Morley and Katznelson, 1965; Gladstones, 1966]. Similarly, *Phalaris* was introduced in very small numbers and it has undergone little adaptive change since, whereas Frankel [1954] also listed several other species that have shown a diversity of adaptive responses since their introduction into Australia.

Many diverse viewpoints were expressed at "The Genetics of Colonizing Species" Symposium held in 1964 [Baker and Stebbins, 1965]. It was clear that no particular genetic system is peculiar to colonizing species. However, the majority of successful colonizers are annuals and many of those are self-pollinating ones [Allard, 1965]. Extensive ecogenetic studies of the colonizing species, *Avena fatua* and *A. barbata*, both inbreeding annuals of the Californian grasslands revealed that *A. fatua* appeared to rely more on genetical variation for adaptation to a variety of environments, whereas *A. barbata* had greater phenotypic plasticity [Jain, 1969].

The two species of the genus *Xanthium* have very successfully colonized large regions of Australia. Since the two species are both proclaimed noxious weeds much of the history of their introduction and subsequent colonization has been documented. *Xanthium strumarium* has been studied extensively in relation to the physiological aspects of flowering and as a result much of its biology is well known. In the literature *Xanthium* species have been considered to be characteristically inbreeding [Love and Dansereau, 1959] and so a population study of these species would enable comparisons with previous studies of other inbreeding colonizing species [Jain, 1969; Allard and Kahler, 1971]. As well there are distinct races of *X. strumarium* in Australia and this would enable comparisons of the success of the races and the strategies employed by them. More

importantly both species occupy highly fluctuating environments, and as well have highly efficient dispersal mechanisms.

It is of interest then to determine what effect the new environments have had on the genetic structure of these colonizing species, whether the genotypes of such species were primarily preadapted and the extent of genetic adaptation after introduction. Is genetic variation both qualitative and quantitative, necessary for successful colonization and if not, what alternative strategies are employed by these species? In particular, in light of the highly fluctuating environments of the two species, is there any evidence of population differentiation with respect to allozyme polymorphisms?

Initially, the research programme centred on the species *X. strumarium* but it was later extended to the other species *X. spinosum*. The genetic structure of the populations were analysed by determining the amount of qualitative and quantitative genetic variation for isozyme polymorphisms and morphological characters respectively. The main results are presented in subsequent chapters of this report.

CHAPTER 2

BIOLOGY

2.1. TAXONOMY AND SPECIATION

The genus *Xanthium* is a member of the tribe Heliantheae of the family Asteraceae [Compositae]. It is closely related to the *Iva* and *Ambrosia* genera. Linneaus [1753] described two species in the genus, *X. strumarium* and *X. spinosum*, which are very distinct morphologically. For example *X. spinosum* plants have 3-branched spines at the nodes below the leaves while *X. strumarium* species have no spines. In addition, as shown in Fig. 2.1, the leaves and fruits of *X. spinosum* are not only smaller but very different in appearance as well to those of *X. strumarium*. However, within the latter species numerous morphological types have been described primarily on the basis of burr morphology and over the last 2 centuries, taxonomists have tended to split *X. strumarium* into many "species". As a result over 50 different species names can be found in the American floras alone [Love and Dansereau, 1959]. Of the many papers on the taxonomy of *Xanthium* the three most important are those of Millspaugh and Sherff [1919], Widder [1923] and Love and Dansereau [1959]. There is agreement that there are distinct Old and New World forms. Millspaugh and Sherff [1919] in a study of the species of *Xanthium* in North America list 20 species, one of which *X. strumarium (sensu stricto)* was "native of the north-temperate and tropical regions of the eastern hemisphere." The others were considered native to the New World. Widder [1923] in his classification of the species of *Xanthium* placed 7 species within the subsection *Orthorrhyncha*

(a)

(b)

Fig. 2.1. Typical plants of (a) *X. strumarium* and (b) *X. spinosum*

(Old World plants) and 12 in the subsection *Camphylorrhyncha* (New World plants). Cronquist [1945], and subsequently Love and Dansereau [1959], put all these "species" back into the original Linnean species, *X. strumarium*. However, Love and Dansereau [1959] provisionally divided this *X. strumarium* L. species into 8 morphological complexes (see Fig. 2.2.). These complexes were defined on the basis of burr morphology. McMillan [1975b] in his extensive photoperiodic studies adopted the classification of Love and Dansereau, and if only to minimize confusion the same taxonomic terminology has been used in the present study. The main reason for placing all complexes in one species is that they can be artificially hybridized.

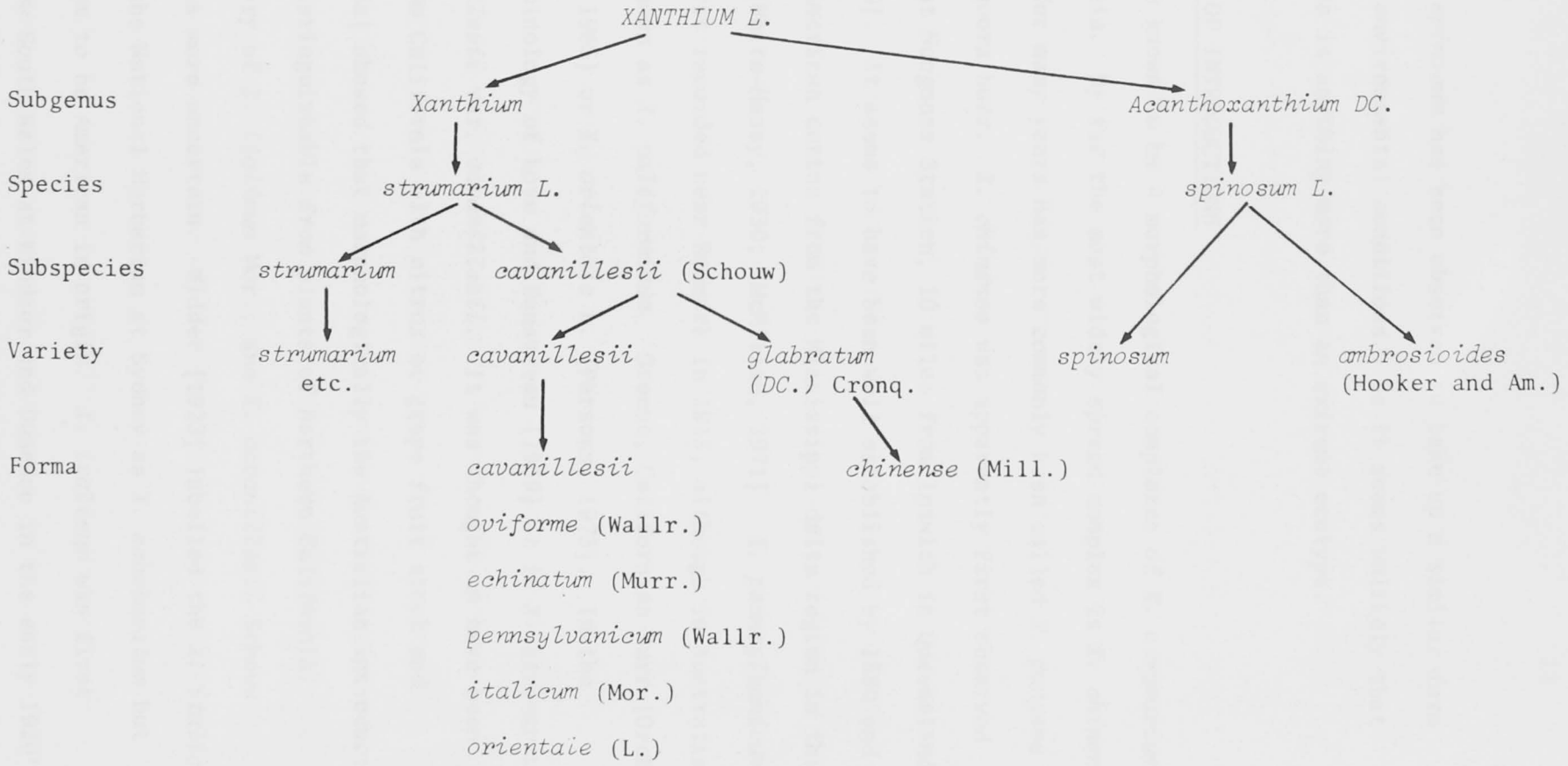
It seems that before man's intervention there was continental isolation of the Eurasian "strumarium" complex from other native American complexes. Love and Dansereau [1959] suggested that the New World complexes originated from *Xanthium cavanillesii* (or a type very similar), which is itself indigenous to South and Central America. Like *X. strumarium*, *X. spinosum* is now a cosmopolitan weed widely distributed throughout the world, but it appears to be native to South America. The connection between the South American centre of dispersal for this genus and the Old World "strumarium" form is not clear. However, Cronquist [1945] stated that "except for a few species of *Ambrosia*, the subtribe Ambrosinae is otherwise exclusively American."

X. spinosum is a stable species with very few varieties recorded subsequent to Linnaeus' original description of the species. One variety, that has been described in several floras is *X. ambrosioides*, which Ridley [1930] described as being similar to *X. spinosum* except that "it is prostrate, with smaller leaves." During the course of

FIG. 2.2.

CLASSIFICATION OF *XANTHIUM*

(Adapted from Love and Dansereau, 1959)



this study *X. spinosum* has been observed to take up a similar form under certain environmental conditions. So it seems unlikely that *X. ambrosioides* is anything more than an extreme ecotype.

2.2. HISTORY OF INTRODUCTIONS

There are known to be 4 morphological complexes of *X. strumarium* within Australia. By far the most widely spread complex is *X. chinense* Mill., which for many years has more commonly been called *X. pungens* Wallr. or Noogoora burr. *X. chinense* was apparently first observed in Australia at Noogoora Station, 10 miles from Ipswich in Queensland [Everist, 1950]. It seems to have been well established by 1880 and came in with American cotton from the Mississippi delta region in the early 1860's [White-Haney, 1930; McMillan, 1971]. *X. pennsylvanicum* Wallr. was first recorded near Renmark in 1915, although in Australia it has been known as *X. californicum.* Greene, Californian Burr [Orchard, 1949; Black, 1965] or *X. orientale* L. [Parsons, 1973]. In the taxonomic terminology of Love and Dansereau [1959] it is *X. strumarium* subsp. *cavanillesii* var. *cavanillesii.* It was thought to have been introduced from California with citrus or grape fruit stock and McMillan [1973a] showed that morphologically the Australian introduction is nearly indistinguishable from plants of northern California.

The history of *X. italicum* Mor., and *X. cavanillesii* Schouw in Australia is more uncertain. Widder [1923] labelled the *X. italicum* specimens in the National Herbarium at Sydney as *X. saccharatum* but considered them to be American in origin. *X. italicum* was first recorded in New South Wales at Windsor and Dunedoo in the early 1920's [Whittet, 1968]. From Herbarium Records, it would seem that *X. cavanillesii* was introduced about the same time, but it is not listed

in any Australian weed manuals. The *X. cavanillesii* complex is indigenous to South America, and McMillan [1975b] has suggested, on the basis of photoperiodic comparisons, that the Australian material originated from Argentina.

X. spinosum was apparently introduced in the tails of horses from Valparaiso, Chile in the 1840's [Maiden, 1920]. It quickly became established at Bathurst as instanced by a letter written from Bathurst in 1861 by Rachel Henning, who stated "that the most objectionable things in the paddock to my mind are the 'Bathurst burrs', which are a real nuisance" [Adams, 1969]. Although indigenous to South America, it has unconsciously been spread by man such that it is now common in warm and temperate regions of the world including throughout the United States, Europe, Western Asia and North and South Africa [Millspaugh and Sherff, 1919].

Because of the confused taxonomy it has been difficult to determine from the early literature to what extent the 8 complexes have colonized different parts of the world. However, recently McMillan [1974d] has found *X. cavanillesii* occurring in Spain and Portugal in some coastal and river situations, while *X. pennsylvanicum* which includes a diversity of types, can be found in Europe and Asia but the restricted type of Australia in sense of *X. californicum* is the only known case outside northern California. *X. italicum* occurs throughout Europe, Asia and the Middle East yet the Eurasian *X. strumarium* complex has been relatively unsuccessful in America [McMillan, 1974c]. *X. chinense* is probably the most widespread or successful colonizer having been found in numerous regions throughout Europe, Asia and Africa [McMillan, 1974d].

2.3. GEOGRAPHICAL RANGES AND ECOLOGICAL HABITATS

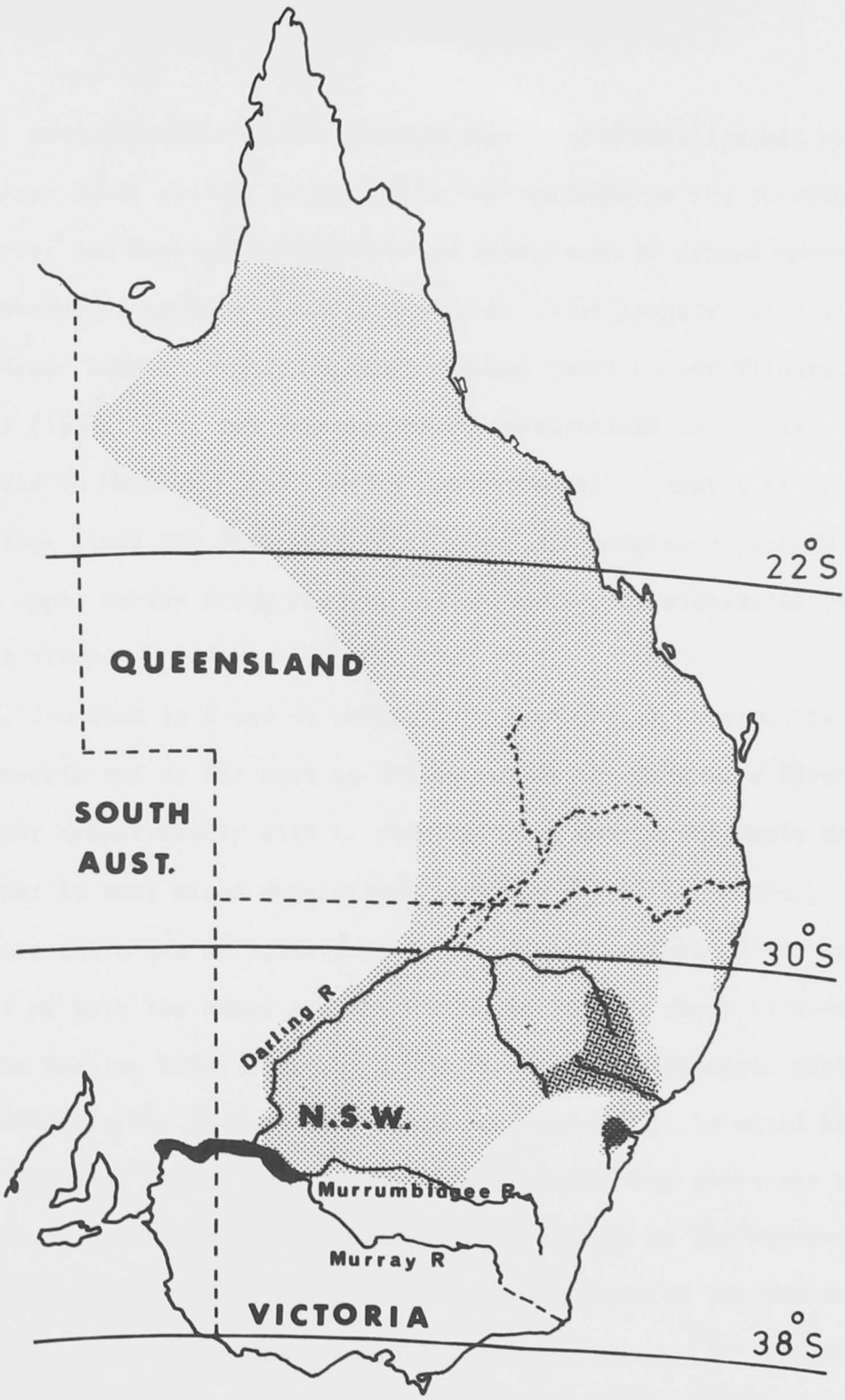
2.3.1. Geographical Ranges

The present distribution of the 4 races of *X. strumarium* in Australia is shown in Fig. 2.3.

These distributions are based primarily on my own field studies, though Herbaria Records and distributions given in the weed manuals of the various States have been taken into account. The geographical areas delineated on the map for the 4 complexes are meant to indicate that substantial populations of the particular complexes do occur within these areas. The geographical ranges of the two species overlap with *X. spinosum* having a more temperate distribution than *X. strumarium*.

X. chinense spread rapidly throughout Queensland and northern New South Wales [White-Haney, 1930]. In the course of field studies it was the only complex detected in Queensland and this has been confirmed by Everist [1974]. The southern limits of its range are in the Murray River region and isolated plants have been reported upstream as far as the Hume Reservoir [Parsons, 1959]. In the colder areas of eastern New South Wales *X. chinense* is very rare. It is not known to occur in Western Australia [Ritchie, 1973] or South Australia [Orchard, 1949] at the present time, and certainly this complex was not detected in South Australia in the present study. Although recorded in Victoria as early as 1911 it never really became established in that State [Parsons, 1973]. *X. chinense* apparently occurs in the north eastern portion of the Northern Territory [McMillan, 1971] but since this area was not surveyed it is not included in Fig. 2.3.

Fig. 2.3. The distribution of the 4 complexes of *X. strumarium* in Australia.


22°S
QUEENSLAND
SOUTH
AUST.
30°S
Darling R
N.S.W.
Murrumbidgee R.
Murray R
VICTORIA
38°S
X. chinense
X. italicum
X. cavanillesii
X. pennsylvanicum

X. pennsylvanicum is distributed along the middle reaches of the Murray River system, occurring as far upstream as the junction of the Murray and Murrumbidgee Rivers and downstream to around Morgan. Field observations have shown that it does exist sympatrically with *X. chinense* between the Murray-Murrumbidgee junction and Mildura, while Parsons [1959, 1973] had reported mixed infestations only from Robinvale to Merbein. The factors (environmental, genetic or otherwise) that limit the distribution of these two complexes particularly in the upper Murray River region, will hopefully be elucidated in the present study.

X. italicum is found in central New South Wales between Newcastle and Tamworth and as far west as Wellington on the Macquarie River. It can occur sympatrically with *X. chinense* over nearly its whole range, such that in some mixed populations there appear to be hybrids, while in others there are definitely not. *X. italicum* occurs on the upper reaches of both the Namoi and the Macquarie Rivers, which flow westward into the Darling River system. *X. cavanillesii* is sympatric with *X. chinense* in the Nepean-Hawkesbury drainage area. In mixed stands the *X. chinense* plants are usually more frequent, but there was no evidence of hybridization in the field. The range of the "cavanillesii" complex is restricted to the banks and flood plains of the Hawkesbury River below the Nepean dam and above Wiseman's Ferry. At Wiseman's Ferry only *X. chinense* plants were observed although *X. cavanillesii* has occurred this far downstream according to the records of the National Herbarium in Sydney.

X. spinosum has spread widely since its introduction and is now present in all States including the Northern Territory. It is found in isolated patches in farmland areas of Western Australia but it is extremely widespread in the Coolgardie-Kalgoorlie gold field area, probably having been introduced by stock transported by rail from the eastern States [Ritchie, 1973]. Rodway [1903] listed Bathurst burr as a weed of wasteland in Tasmania since the turn of the century. It does occur in most of the settled areas of South Australia [Orchard, 1949] but it is in the irrigation areas associated with the Murray River that it is predominately found. However, probably 80% of the *X. spinosum* plants in Australia in any one season occur in the southern and central regions of eastern Australia from central Victoria to the northern half of New South Wales. It is relatively infrequent in the eastern coastal regions but common on the western slopes and plains of New South Wales [Whittet, 1968]. Of course, being a proclaimed noxious weed in all States, it is unclear how much the present distribution is a reflection of weed control measures rather than environmental or genetic limitations.

The species can also be found through southern Queensland, but is rarer further north such that isolated plants rather than population stands are the norm north of the Tropic of Capricorn.

2.3.2. Ecological Habitats

Although the two species cover large geographic areas, within these regions they are located in rather narrow ecological habitats. These are the banks of water courses and the associated flood plains, disused cultivations and roadsides. These habitats are characteristically

open, very much non-climax communities with very few permanent species. On the whole there is a very low percentage ground cover of perennials in these habitats. Moreover *Xanthium* species are the first summer annuals to occupy these sites, and they appear to become established before effective interspecies competition can occur. Field observations suggest that normally in these environments, interspecific competition, is nowhere as intense as is typically found in closed communities.

In the more favourable coastal regions *X. chinense* forms regular stands at the same sites every year. However, in the drier more unstable western areas there is immense variability in the size and regularity of occurrence of populations, especially along river systems. There is a continually changing mosaic of populations both in time and space within this actual niche. Cultivation and roadside areas are favourable for *Xanthium* species primarily because they have become open habitats as a result of disturbance of man, whereas river banks and flood plains although also open, are typical of their native habitat.

In Australia *X. strumarium* primarily is situated along river courses and flood plains while *X. spinosum* is far more common in disturbed cultivations and farmland. Over 60 population sites were sampled in the course of this work and details of those that have been used in experimental studies are shown in Table 2.1. A random sample of plants was collected at each site and where possible a half-sib family of at least 10 fruit was collected from each of these plants. The number of plants sampled in each population is given in Table 2.1. Love and Dansereau [1959] described *X. strumarium* in indigenous habitats of North America as "primarily a beach plant which prefers open habitats and succumbs to crowding." They pointed out

TABLE 2.1.

LIST OF *XANTHIUM* POPULATIONS

Population	Number	Site Position		Species	Date collected	Number of plants	Site description
		Latitude	Longitude				
Wiangaree	6	28° 29'	152° 58'	*chinense*	28/ 6/72	61	Bank of a tributary of the Richmond
Oxford Downs	11	21° 50'	148° 41'	*chinense*	2/ 7/72	162	Banks and environs of dry creek
Fernless	13	23° 52'	148° 08'	*chinense*	3/ 7/72	127	Banks of a tributary of the Comet River
Revilo	17	26° 35'	148° 03'	*chinense*	5/ 7/72	125	Dry sandy bed of the Manaroa
Maude	22	34° 28'	144° 55'	*chinense*	13/10/72	125	Overflow lagoon of Murrumbidgee
Deniliquin	23	35° 32'	144° 55'	*spinosum*	14/10/72	121	Disused paddock
Waikerie	25	34° 12'	140° 00'	*spinosum* *pennsylvanicum*	10/11/72	16 69	River flats of Murray
Morgan	26	34° 03'	139° 41'	*pennsylvanicum*	10/11/72	150	Banks of Murray
Wilpena	27	31° 24'	138° 43'	*spinosum*	13/11/72	131	Dry, sparse roadside
Swan Hill	31	35° 20'	143° 33'	*spinosum*	1/ 2/73	143	Banks of the Murray River

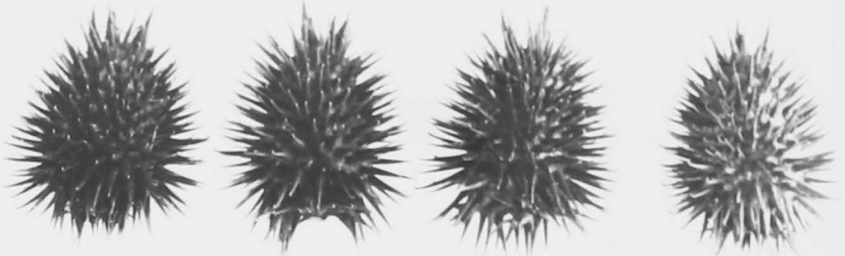
Numurkah	33	36° 04'	145° 27'	*spinosum*	2/ 2/73	102	Cultivated paddock and orchard
Dubbo	34	32° 15'	148° 35'	*chinense* *spinosum*	20/ 8/73	174 41	River flats of the Macquarie
Mungindi	38	28° 59'	149° 00'	*chinense*	22/ 8/73	195	Banks of the Barwon River
Brewarrina	42	29° 05'	146° 00'	*chinense*	23/ 8/73	189	Banks of Barwon
Coolgardie	43	31° 03'	121° 09'	*chinense*	1/ 5/74	180	
Windsor	44	33° 36'	150° 49'	*chinense* *cavanillesii*	13/ 8/74	86 75	Ridge of river flat of Hawkesbury
Muswellbrook	46	32° 15'	150° 52'	*italicum*	14/ 8/74	157	Above bank of Hunter - heavily grassed
Raymond Terrace	50	32° 45'	151° 44'	*chinense*	17/ 8/74	145	Flood plain of Hunter
Sandy Hollow	52	32° 20'	150° 34'	*italicum*	4/12/74	100	Bare gravel sand - bank of Goulburn River
Binnaway	53	31° 33'	149° 24'	*chinense*	5/12/74	100	Ridge of bank of Castlereagh
Darlington Point	55	34° 35'	146° 00'	*chinense*	14/ 4/75	90	Overflow area of Murray
(1)	56)			*spinosum*	15/ 4/75	72	Disused horse paddock *spinosum* dominant
Hay (2)	57)	34° 30'	144° 52'	*spinosum*	15/ 4/75	84	Dry, grey clay soil - 200 yd from river
(3)	58)			*chinense*	16/ 4/75	102	Bank of Murrumbidgee
Mildura	59	34° 11'	142° 09'	*pennsylvanicum*	15/ 4/75	100	Sandy river flats of Murray

that cockleburs are uncommon in mountainous areas but that the number of species are profuse in areas disturbed by man e.g. waste places, roadsides, railway banks etc. McMillan [1970] obtained most of his *Xanthium* collections from river bottoms in Texas. Likewise, earlier in the century, Symons [1926] wrote of several "species" frequenting the shores of the St. Lawrence River. Kaul [1965] listed similar habitats for the 4 varieties of *X. strumarium* in India. Thus the habitats in Australia in which *X. strumarium* is now found are very similar to those occupied by this species in other parts of the world. There are in effect 2 types, both of which are open, with 1 being the natural river course or beach situation and the other, areas disturbed by man. Moreover, all 4 complexes of *X. strumarium* have been in these same ecological niches since their introduction into Australia. The 2 least successful races are almost entirely restricted to river bank environments although in the past, stands of *X. pennsylvanicum* have been reported away from the Murray River [Orchard, 1949; Parsons, 1959].

The nature of the native habitats of *X. spinosum* is uncertain, but in its colonization of many areas of the world it appears to have become established in similar habitats to those in Australia [Ridley, 1930; Orchard, 1949]. It should be emphasized that both *X. spinosum* and *X. chinense* have been in Australia nearly twice as long as the other three species.

Fig 2.4 Fruits of Xanthium

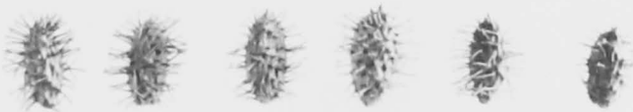
X.chinense



X.cavanillesii

X.italicum

X.pennsylvanicum



X.spinosum L.

2.4. BIOLOGY

Both species are monoecious annuals with clusters of inconspicuous male and female flowers in the leaf axils and at the end of the branches. The male flowers are in clusters along slender branchlets while the female flowers are lower down on the same branchlet as well as in clusters in the leaf axils. In each male flower head there are 5 anthers. These anthers range in colour from brown to black in *X. spinosum*, black in *X. chinense* and yellow often with a red central streak in the other 3 races of *X. strumarium*. F_1 plants from crosses between *X. chinense* and any of the other races have black anthers.

X. strumarium has an erect growth form, and has been observed to vary from 15-250 cm in height. Plants can be single stemmed or at the other extreme much branched and widely spread as in Fig. 2.1. (a). In the field the 4 races can be distinguished not only by their fruit but also on leaf morphology and stem colour. Differences in leaf morphology have been detailed by McMillan [1975b]. From Fig. 2.4. the fruits of the races of *X. strumarium* and *X. spinosum* can be seen to be quite distinct. Taxonomic descriptions of the fruits of the complexes are given by Love and Dansereau [1959]. The colour pigmentation of the stems and petioles of *X. chinense* is normally dark red, while those of *X. cavanillesii* are faintly pink and those of *X. italicum* and *X. pennsylvanicum* are green. Field and experimental observations suggest there are genetic differences between races for this character but that the expression of this character is markedly affected by environmental conditions.

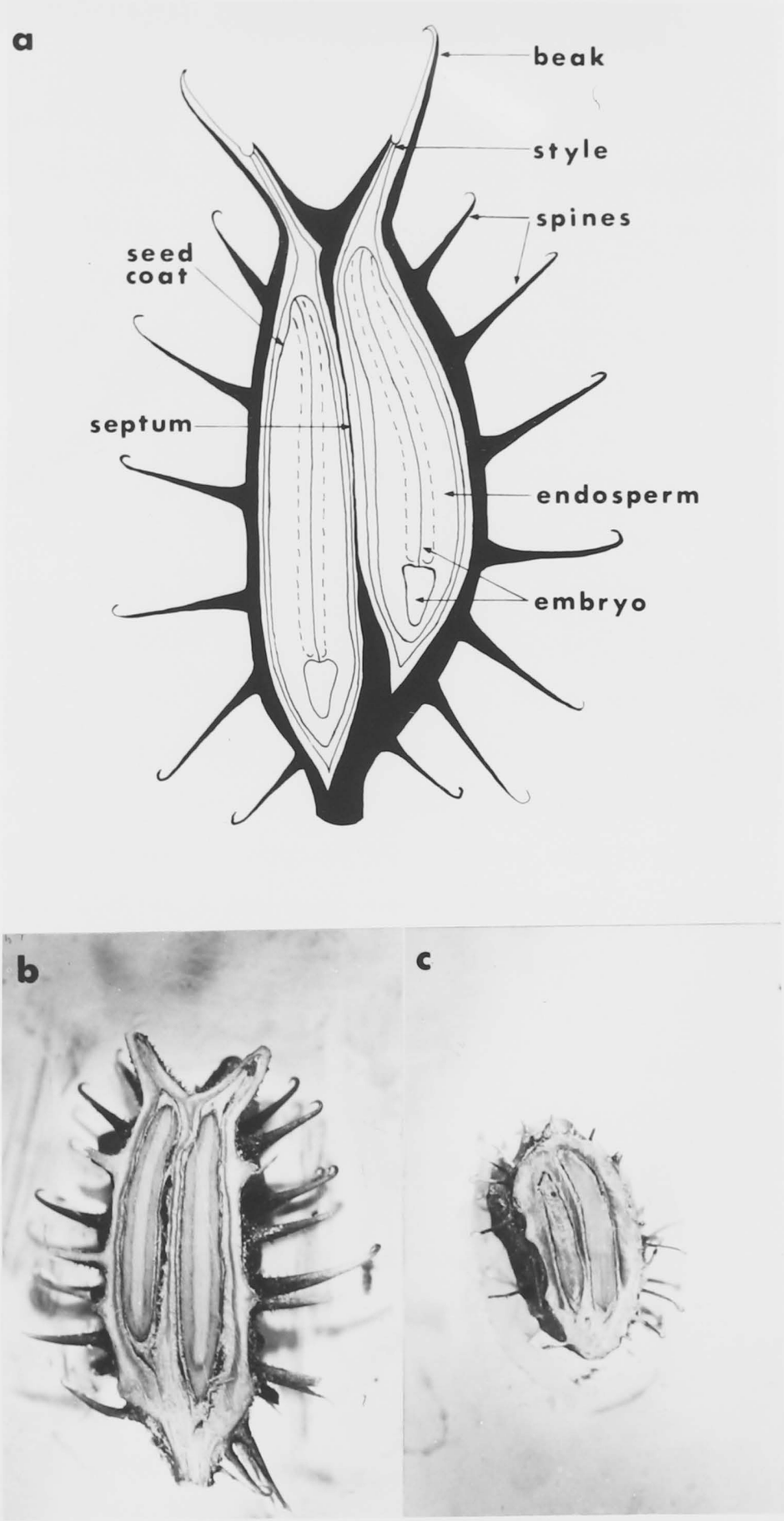
X. spinosum plants can grow up to 120 cm in height. They also can be single stemmed structure but more commonly have several branches (Fig. 2.1. (b)). The burrs are much smaller than those of

Fig. 2.5. Longitudinal sections of *Xanthium* fruit

(a) Diagram showing the principal parts of a fruit of *X. strumarium*.

(b) A fruit of *X. italicum*

(c) A fruit of *X. spinosum*


a
beak
style
spines
seed coat
septum
endosperm
embryo
b
c

X. strumarium being from 7-10 mm long, light brown in colour with many thin spines and normally one or two straight terminal spines.

The fruits of both species have dimorphic seeds with the lower seed larger than the upper one (Fig. 2.5.) Crocker [1906] confirmed that the lower seed germinates more readily than the upper one. The data indicated that the dormancy of the upper seed was due to an exclusion by the seed coat of oxygen. Dormancy may be said to occur, when seeds in conditions normally favourable for germination (adequate moisture and suitable temperature) do not germinate. Subsequently, this dormancy was examined by several workers, who on the whole accepted Crocker's explanation of the phenomena [Schull, 1911; Schull and Davis, 1923; Davis, 1930; Thornton, 1935]. They found that the dormancy could be overcome by high O_2 partial pressures or by high temperatures and that removal of the seed coat leads to germination of the embryo.

However, McHargue [1921] and Symons [1926] claimed that the dormancy was broken if seeds were removed from the fruit and therefore the burr was the factor in the retardation in growth of the upper seeds in nature. This explanation implied as McHargue [1921] stated that "there is no inherent property contained in the embryos of the small seeds of this species, which is the cause of delayed germination." In other words, the dormancy is not genetically controlled by the embryo but by the genotype of the mother plant which determines fruit structure. On the other hand, dormancy was induced in intact upper and lower seeds by placing them in various gas mixtures (in the absence of oxygen) and this apparent embryo dormancy could be removed by moist storage of the intact seeds at 5°C [Thornton, 1935].

Wareing and Foda [1957] described the presence of 2 water soluble inhibitors in both upper and lower seeds of *X. pennsylvanicum*. Seed coats were impermeable to the inhibitors but the latter are rapidly leached out of naked embryos. Also there was no evidence of significant difference in inhibitor content between the upper and lower seeds. Both upper and lower seeds were dormant immediately after harvesting, but after a period of ripening the lower seeds emerged from dormancy, yet this did not correlate with any changes in inhibitor levels. They concluded that the dormancy was attributable to the lesser ability of the embryo of the smaller seed to oxidize a water soluble inhibitor.

A comparison of the forces generated by germinating *Xanthium* seeds and the restraining forces of the seed coat indicate that "whereas the thrust developed by non-dormant seed is adequate to cause testa rupture that developed by dormant seeds is not" [Esashi and Leopold, 1968]. As well, the thrust generated was less in anaerobic than in aerobic conditions as it might be expected that any situation reducing oxygen diffusion would lead to reduced thrust. To verify Crocker's explanation of the dormancy the actual rates of oxygen consumption and diffusion of individual seeds should be determined. If the rate of oxygen consumption is much less than the rate of oxygen diffusion into the seed then oxygen impermeability of the seed coat is probably not the cause of the dormancy. Porter and Wareing [1974] found that (1) the moist upper and lower seed coats do not differ significantly in the permeability to oxygen (2) furthermore the diffusion and consumption rates are about the same for upper and lower seeds and (3) more significantly the rate of oxygen diffusion through the seed coat is higher than the rate of consumption by the embryo. These

results do not support the view that dormancy is due to seed coat restriction of O_2 diffusion. In an attempt to reconcile these findings with the other two theories, Porter and Wareing [1974] suggested that the dormancy of the freshly harvested seeds could be due to inhibitors, whereas that in after-ripened upper seeds may be due to the inability of the embryo to rupture the seed coat.

X. spinosum despite its smaller fruit and correspondingly smaller but dimorphic seeds (Fig. 2.5. (c)) also exhibits a dormancy of the upper seeds in the field [Orchard, 1949; Parsons, 1973]. It is not known whether the mechanism of dormancy in *X. spinosum* is the same or different to that of *X. strumarium*. These findings of Wapshere [1974] and Mann [1965] demonstrated the differential germination of the two seeds of *X. chinense* in the Australian field situation. As Crocker [1948] has pointed out, lower seeds will germinate at 21°C while higher temperatures (above 33°C) are required for upper seed germination. Hence in the field it appears that the upper seed is dormant until the summer soil temperature has become sufficiently high, although by this stage in the season moisture and soil conditions could be unfavourable. Mann [1965] found that *X. chinense* fruit covered by 6 inches or more of soil did not produce plants and that practically all the seeds under these conditions had decomposed within a period of 4-5 years. Field observations indicated that both species can grow successfully under a wide range of edaphic conditions.

X. strumarium is a short day plant requiring a minimum number of hours of uninterrupted darkness for the initiation of flowering [Hamner and Bonner, 1938]. The photoperiodic control of flowering in *Xanthium* has been reviewed by Salisbury [1969]. The critical night length (minimum number of hours of darkness required to initiate flowering) increases with decreasing latitude for North American

populations such that plants in the northern most population have a 7.75 hour critical night length whereas the southern populations of central Texas required 10.5 hour dark periods [Ray and Alexander, 1966].

In a subsequent study of *X. strumarium*, a range in critical night length from 9.5 hours in Northern Texas to 10.75 hours in south eastern Texas and parts of Mexico was documented [McMillan, 1970]. Furthermore, the findings pointed to a combination of critical night length and ripeness to flower (maturity) responses as being the basis of reproductive adaption of this species in its native habitats over a wide latitudinal range of diverse climates. Three different photoperiodic types within the *X. strumarium* of India have been reported [Kaul, 1965]. One of these, a day-neutral form was in fact the Old World "strumarium" complex, and the others probably were hybrids between "strumarium" and New World introductions [McMillan, 1974d]. The critical night length requirements for floral induction for the 8 morpho-physiological complexes of *X. strumarium* and the range of adaptation within these complexes for native American and many introduced populations of the world are summarized in Table 2.2. Data in this Table are from the work of McMillan [1973a, 1974b,c,d,; 1975b]. The range of photoperiodic diversity among introduced populations in various parts of the world is nearly as broad as among indigenous populations in North America. Within the Old World "strumarium" complex the photoperiodic diversity only ranges from day neutrality to critical night requirements of 8 hours or less. McMillan [1974d] considered that part of the wide range of photoperiodic adaptation of the present Old World *Xanthium* populations probably resulted from hybridization between indigenous and introduced

TABLE 2.2.

RANGE OF PHOTOPERIODIC ADAPTATION IN THE 8 MORPHO-PHYSIOLOGICAL COMPLEXES OF *X. STRUMARIUM* (ADAPTED FROM McMILLAN, 1975b)

	Range of critical night lengths (hr)		
Complex	Old World	New World	Australia
"strumarium"	0, 7.5-8.0, 9-9.25		-
"chinense"	9.75-10.75	8.0, 8.5-10.5	10.5
"italicum"	0, 7.5-9.25	7.75-10.75	10.0
"cavanillesii"	9.5 - 9.75	9.5 -10.25	9.5
"pennsylvanicum"	-	8.00- 9.25	9.25-9.5
"echinatum"	7.5 - 8.25	0,7.5 - 8.5	-
"orientale"	7.5-8.0, 8.5-8.75	-	-
"orientale"	9.5 - 9.75		
"oviforme"	-	8.0 - 8.5	-

species, but that primarily the range was imported from America. The 4 complexes which have colonized Australia have different critical night length requirements for flowering [McMillan, 1975b] - see Table 2.2. From the critical night lengths of the 4 Australian complexes McMillan extrapolated to the probable sites from which these introductions originated.

Field studies are in agreement with the different photoperiodic adaptations of the 4 Australian introductions. Thus in the Riverina, while *X. pennsylvanicum* and *X. chinense* can be found growing sympatrically they are effectively isolated from each other since *X. pennsylvanicum* flowers in late January/early February but *X. chinense* does not begin to flower till the middle of March. Similarly, along the Hawkesbury

River system *X. chinense* and *X. cavanillesii* grow sympatrically but the former flowers from March on and the latter at the beginning of February. On photoperiodic grounds (at least from McMillan's data), there has been no adaptation within complexes since introduction to Australia. Not surprisingly, hybrids between *X. italicum* and *X. chinense* have been detected photoperiodically from the central region of New South Wales but whether hybrids as such were introduced into Australia is unclear.

Single gene control of flowering in rice has been shown [Chandraratna, 1955], whereas in the short day species *Solidago sempervierens* many genes are apparently involved [Goodwin, 1944]. Findings from hybridization studies between complexes of *X. strumarium* have indicated that photoperiodic control of flowering is quantitatively controlled and inherited independently of morphology and ripeness to flower response [McMillan, 1974a, 1975a]. However, as will be discussed in detail in a later chapter, the genes controlling floral induction can be of some importance in determining the phenotypic expression of other characters such as height, number of fruit, leaf length etc.

X. spinosum has been shown not to have a qualitative short-day requirement and is in fact day neutral [Lona, 1946]. This has been confirmed in the present study but a quantitative response has been detected such that the time to flower initiation is less, the longer the night length (up to 12 hours darkness). Field and nursery observations have pointed to a maturity factor also being involved in flowering. It appears that plants do not flower till they have reached a certain stage of development and that is influenced by environmental conditions.

Members of the subtribe Ambrosinae have become adapted to wind pollination, and differ from the rest of the Asteraceae with much reduced corollas and free or nearly free anthers [Cronquist, 1955]. Despite this, *Xanthium* species appear to have always been described in the literature as highly inbreeding species [Love and Dansereau, 1959].

Both *Xanthium* species have nearly world wide distributions as a result of man's activities. The transport by man of stock, crops and other material between continents has enabled the introduction of the species to various areas of the world. Fruits and seeds of plants can be dispersed by many mechanisms. Plants, like *Xanthium*, which are dispersed by more than one agent can be said to exhibit a plasticity of dispersal mechanisms. Many such cases are cited by van der Pijl [1972]. In Australia *Xanthium* is dispersed primarily by water and by animals. Water is the main means for *X. strumarium* while stock is more important for dispersal of *X. spinosum* fruits. As far back a 1930, Ridley [1930] stated that "the combination of animal and water dispersal of these plants is certainly the cause of the very wide dispersal of *Xanthium* all over the world." Perhaps more importantly this implies that there is considerable gene flow within the geographic ranges in Australia, especially for the races of *X. strumarium*. This would be in contrast to the very low gene flow between plant populations argued for by Bradshaw [1972]. If this gene flow were appreciable, it would reduce the interpopulation genetic differentiation for the predominately river bank *X. strumarium* compared to *X. spinosum*, which occurs more frequently in cultivated areas. There is also a close interaction for *Xanthium* between dispersal by water to the actual favourable sites for colonization, namely river banks and subsequent moisture conditions suitable for successful colonization.

Ecological and other aspects of the biology of *Xanthium* are discussed later in the appropriate sections. The two main reasons for placing the types of *X. strumarium* within the one species were that (1) it was the currently accepted taxonomic terminology and (2) the "species" can be crossed quite readily in the nursery, even though most of the races are effectively isolated in the field from one another due to different photoperiodic requirements. On many biological grounds, as will become evident from this study, the races could be considered different species.

Only partial fertility of F_1 hybrids between subspecies of *X. strumarium* was shown by McMillan [1973b]. In the present study despite several attempts, including the use of solvent treatments [Hamilton - personal communication] on both pollen and stigmas, *X. spinosum* and *X. strumarium* could not be successfully hybridized. Bitter [1908] was also unable to cross the two species successfully. Several workers have looked for apomixis but there was no evidence of it [Love and Dansereau, 1959; Symons, 1926]. Removal of male flowers and isolation of the plants from other pollen sources leads to fruit development without concomitant seed formation. The possibility of facultative apomixis has not been eliminated.

CHAPTER 3

QUALITATIVE VARIATION

3.1. GENETIC VARIATION IN NATURAL POPULATIONS

The levels of genetic variation in natural populations of plants and animals were largely unknown until the development of gel electrophoresis enabled organisms to be screened for enzyme polymorphisms. The first population studies, using genetically determined isozyme variants (allozymes) as markers, demonstrated extensive genetic variation in *Drosophila* [Lewontin and Hubby, 1966] and man [Harris, 1966]. Lewontin and Hubby [1966] showed that in *D. pseudoobscura* 30% of the loci are polymorphic and about 11.5% of loci are heterozygous per individual. As has already been discussed these are undoubtedly underestimates of the total genetic protein variation [Lewontin, 1974], and in fact, recent studies have demonstrated a further class of variants, namely temperature sensitive alleles [Bernstein *et al*, 1973]. Lewontin [1974] reviewed many of the allozyme population studies of animals, especially those on *Drosophila* species, and calculated that the median proportion of polymorphic loci was 30% with the median heterozygosity per individual at 10.6%. There is a large range in genic heterozygosity, however, with tetrapods, including man, having estimates around 6% while those for invertebrates are higher - in the region of 15% [Selander and Kaufman, 1973b].

Recent studies of a diversity of organisms, such as *Limulus* [Selander *et al*, 1970), the house mouse [Selander and Yang, 1969] *Fundulus heteroclitus* [Mitton and Koehn, 1975], adriatic lizards

[Gorman *et al*, 1975), parthenogenetic moths [Lokki *et al*, 1975] and plants [Allard and Kahler, 1971] have confirmed that extensive genetic variation in natural populations is the rule rather than the exception. The marked geographic uniformity at allozyme loci in natural populations of several organisms, in particular those of *Drosophila*, has been interpreted as indicating that selection was responsible for the maintenance of isozyme polymorphisms [Ayala *et al*, 1972]. However, it has been recently shown [Ohta, 1975; Latter, 1975] that the gene frequency data from population surveys of *Drosophila* species can be accounted for by mutation-selection balance. Interpopulation differentiation has occurred in *Acris crepitans* [Dessauer and Nevo, 1959], in which at some loci different alleles are fixed in different regions, and in European house mouse populations [Selander *et al*, 1969] though in the latter case it could be due to the presence of two separately evolving subspecies within the species. On the whole, isozyme studies of animals indicate that marginal populations are as heterozygous as central ones, but Gorman *et al* [1975] present data suggesting that certain island populations of lizards have smaller amounts of genetic variation than mainland populations.

There has been much controversy about whether isozyme variants have a selective role [Clarke, 1970; Stebbins and Lewontin, 1972] or whether they are in fact neutral [Kimura and Ohta, 1971]. Usually some form of balancing selection is invoked as the mode of maintenance of isozyme polymorphisms, though other selective mechanisms are possible and have been reviewed by Karlin and McGregor [1972]. The classic example supporting the "selectionist" viewpoint is the clinal change in esterase gene frequencies in catostomid fishes in response to a temperature gradient [Koehn, 1969]. Several reports on the differences

in allelic properties at ADH loci in *Drosophila* and maize [Gibson and Miklovich, 1971; Marshall *et al*, 1973; Morgan, 1975] suggest different adaptive roles. Alcohol dehydrogenase in *Drosophila* and sickle cell haemoglobin are examples of electrophoretic variants with effects on fitness.

Studies of allozyme polymorphisms in plant populations have revealed similar amounts of genetic variation as found in other organisms. Marshall and Allard [1970a, b] found in populations of *A. barbata* and *A. fatua* that the proportion of polymorphic loci was about 31% and 54% respectively with 3% of the loci heterozygous per individual, but that *A. fatua* had higher levels of genetic variation. Comparison of calculated fixation indices ($\hat{F}$) and theoretical inbreeding coefficients [F_n] suggested that selection favouring heterozygotes was of some importance in maintaining the polymorphisms in both populations.

An extensive survey of the genetic differentiation in 14 populations of *A. barbata*, an introduced inbreeding annual, showed that the Californian gene pool is similar overall in composition to the ancestral Mediterranean gene pool, but there is marked differentiation between Californian populations with those in one region markedly monomorphic and identical to each other, but those in a second region are "all highly differentiated from each other" [Clegg and Allard, 1972]. Selection favouring a single genotype in arid habitats and the contrasting genotype in mesic habitats appeared to be the primary force responsible for the observed patterns of monomorphism and polymorphism in Californian populations. Allard *et al* [1972] demonstrated from multilocus analyses that large gametic phase disequilibrium for both linked and unlinked loci occurred with two balanced five-locus gametic types predominating - one being associated with mesic conditions, the other with xeric habitats. However, Lewontin [1974] pointed out that with the

very low outcrossing rate in *A. barbata* there is non-random association of loci and he suggested that the geographic distribution is a linkage effect "with one or more unknown loci that are under selection" such that there is no direct relationship at all necessarily between the 5 loci and the environment.

Clegg, Allard and Kahler [1972] analysed two experimental barley populations over several generations and found that in this highly inbreeding species (rate of outcrossing t = 0.0057) selection in both populations directs the genetic material into the same coadapted gene complexes, in which are incorporated not only tightly linked loci, but unlinked ones as well. In the outcrossing grass, *Lolium multiflorum* (t > 0.9) the gene frequencies at 4 loci in three populations over 2 years were very similar, and moreover the genotypic frequencies agreed with Hardy-Weinberg expectations indicating no excess of heterozygotes [von der Pahlen, 1969]. More recently marked microgeographic variation at 3 out of 4 loci at a single population site of the inbreeder *Bromus mollis* (t = 0.08) has been demonstrated [Brown *et al*, 1974]. As well it was found that there was an excess of heterozygotes at nearly all the marker loci but this occurred largely in the more xeric sites rather than the mesic ones. Similar microgeographic differentiation over very short distances was found in *A. barbata* [Hamrick and Allard, 1972].

Thus findings on inbreeding grasses have generally shown an excess of heterozygotes to that expected on the basis of the mating system alone. Moreover, indications are that loci may be tied together into coadapted gene complexes as a result of the breeding system, but to date this has only been shown in one species, *A. barbata*. In grasses, monomorphism is the exception rather than the rule and inbreeders are nearly as variable as outbreeders, but there is a suggestion of greater geographic differentiation in inbreeders compared to outbreeders.

Allozyme population studies on plants, other than grasses, have been characterized by a lack of estimation of all the genetic parameters, especially the outcrossing rate. Early studies included those on *Leavenworthia* [Solbrig, 1972], *Taraxacum officinale* [Solbrig, 1971], *Lycopodium lucidulum* [Levin and Crepet, 1973] and *Stephanomeria* [Gottlieb, 1973c].

The self-compatible species in the genus *Leavenworthia* show less variation in isozyme patterns, and less within and between family variation, than the obligate outbreeders. However, the genetic basis of the isozyme bands in these species is still unclear. In the diploid annual, *Stephanomeria exigua*, the obligate outcrossing subspecies *coronaria* had 8 out of 13 loci polymorphic at one population site, while an apparently new self-compatible subspecies "Malheurensis" was fixed for the most common alleles at 11 out of 13 loci in the single population of this species [Gottlieb, 1973c]. Data suggest that little allozyme differentiation is necessary in the initial speciation process at least for these annual plants. In 16 New England populations of the slowly evolving *Lycopodium lucidulum* the proportion of polymorphic loci per population averaged 0.10, while the mean heterozygosity per individual averaged a low 0.06 [Levin and Crepet, 1973]. There was little interpopulation differentiation compared to that found in inbreeding grasses, but it can reproduce sexually and asexually with the latter predominating.

For the apomictic dandelion, *Taraxacum officinale*, there are quite large amounts of allozyme variation between clones [Solbrig, 1971], while in a population of *Oenothera biennis*, a permanent translocation heterozygote, 26% of the loci are polymorphic, but the proportion of heterozygous loci per individual is very high - 26% [Levin *et al*, 1972]. In fact, nearly all the plants have the same genotype. Recently more

extensive studies of populations of *O. biennis* [Levin, 1975b] and strains of other species of the genus *Oenothera* [Levy and Levin, 1975] have been made. For 44 Illinois populations of *O. biennis* 20% of the loci were polymorphic, but 59% of the populations had 1 genotype, 27% had 2 and the rest 3-5 genotypes. These studies suggested that, in contrast to the situation in the colonizing grass species, the population structure of this inbreeding weed is one of genetic uniformity. The mean heterozygosity in Illinois populations of *O. biennis* is 4.5%, while for the other two self-pollinating related species *O. strigosa* and *O. parviflora* the heterozygosities are 3% and 15% respectively. These species, because of their chromosome arrangements, have in effect a single coadapted gene complex with maximum linkage.

In the perennial *Liatris cylindracea* the percent polymorphic loci was 5.6 and the mean heterozygosity 6% [Schall, 1974 quoted by Levin, 1975]. In a comparison of the outbreeder *Phlox drummondii* and the self-compatible *Phlox cuspidata* Levin [1975a] found that the obligate outbreeder is more polymorphic and heterozygous than the predominant inbreeder, but had less interpopulation differentiation. Moreover, both of these annual species displayed heterozygote deficiency and it was more marked in *P. cuspidata* than in *P. drummondii*. The actual heterozygosity levels are unusually low especially for the outbreeder. The mean proportion of heterozygous loci ranges from 8-16% for various annual species of *Clarkia* [Gottlieb, 1973b], is 35% in *Lupinus texensis* and 10% in *L. subcarnosus*, but is 20% in both *Hymenopappus scabiosaeus* and *H. artemisiaefalius* [Babbel and Selander, 1974]. These latter figures come from a study relating the degree of genetic variability in the 2 pairs of species to their ranges of edaphic tolerance. For the two herbaceous *Hymenopappus* species

the levels of genetic variability are similar, whereas in *Lupinus* there is an inverse relationship between level of genetic variability and degree of edaphic restriction.

As distinct from studies on annual plants there are no extensive surveys of allozyme variation in tree populations. However, the few studies of polymorphic loci have shown high levels of genetic variation in several species. Rudin *et al* [1974] showed geographic differentiation in 3 populations of *Pinus sylvestris* and although there was a general deficiency of heterozygotes it was actually significant at only 1 locus in 1 population. Accurate estimates of mating system parameters in tree populations are rare. Squillace [1974] listed outcrossing rates for some conifers but these were largely based on morphological characters. Brown *et al* [1975] gave an overall estimate of 24% self-fertilization in *Eucalyptus obliqua* with evidence moreover of marked genetic differentiation between all 4 sites with all loci contributing to this. Recently, considerable genetic differentiation between 4 sites at 4 allozyme loci in the predominately outcrossing *E. pauciflora* has been found [Phillips - personal communication].

Although allozyme studies of plants are not as extensive or as exhaustive as those on animals it is clear that high levels of genetic variation comparable to that found in other organisms is the norm in plants. No examples of species completely monomorphic over their whole range have been detected, although isolated populations of "new" species of *Clarkia* and *Stephanomeria* are almost monomorphic.

On the other hand, there are a few reports of the absence of genetic variation in animal species. There are several studies showing low genetic variation in Fossorial rodents [Nevo *et al* 1974 and citations therein] with the explanation that it is due to selection for homozygosity in a narrow constant ecological niche. The mean

number of heterozygous loci per individual is in the range of 3-7% for the 20 species examined. Lewontin [1974] has reviewed the early literature on low genetic variation and claimed that because of the low number of loci scored none of the species are significantly more homozygous than the more extensively studied *Drosophila*, but Nevo's latest findings cannot be subject to this objection. The self-fertilizing land snail, *Rumina decollata*, a native of the Mediterranean region, exhibited no genetic variation at 25 loci within or among populations in the United States [Selander and Kaufman, 1973a]. Two explanations were possible. Either there was selection for a uniquely adaptive monogenic genotype or there was no variation in the original introduction from Europe. Snyder [1974] found no allozyme variability at 24 loci for 3 bee species but few individuals were studied.

Finally, allozyme population studies can provide data on the extent of genetic differentiation occurring during and subsequent to the speciation process. Do closely related plant species have the same loci and are the allelic frequencies similar? Is it possible from the genotypic frequencies at a locus in a plant to assign the plant to a particular species? Work by Prakash [1969] and Ayala and Powell [1972] point to the presence of 4 diagnostic loci out of 39 for *D. pseudoobscura* and *D. persimilis*. A locus is defined as diagnostic if, when the unknown individuals are placed in that species in which their genotype was more frequent, the probability of error is small ($< .01$). Undoubtedly, this is looking at speciation well after the event yet on the whole the data suggest that large amounts of the genomes are identical immediately after speciation. Only a few allozyme studies of closely related plant species have been done [Levin, 1975a;

Gottlieb, 1973b, c]. Perhaps very important to this discussion is the rapidity of the speciation process, since it could be envisaged that slowly evolving species could build up quite a number of genetic differences over time.

3.2. MATERIALS AND METHODS

3.2.1. Isozyme techniques

The technique of starch gel electrophoresis was developed by Smithies in 1955. It soon found application in enzyme studies, in which histochemical methods were used to visualize the position of enzymes in gels. Markert and Moller [1959] coined the word isozyme to describe the multiple enzyme bands, which appeared after staining for a particular enzyme. Mediums other than starch, such as cellulose acetate strips, agar and acrylamide have also been found to be suitable for separating the multiple molecular forms of enzymes.

In this study both horizontal starch and acrylamide electrophoresis were employed. The basic techniques used were those of Brewbaker *et al* [1968]. The starch gels (13%) were made from hydrolysed starch (Connaught Laboratories, Toronto, Canada) in 330 ml of buffer. Three different gel buffer systems were used (see Appendix A for details of buffer compositions). The one used for the majority of enzymes consisted of 10:1 mixture (300 + 30 ml) of buffer I and buffer II (Final pH 8.4). Of the other 2 gel buffers one was a .005M histidine, pH 8.0 (buffer III) following the method of Brewer and Sing [1970] and the other 330 ml of buffer IV [after Shaw and Prasad, 1970].

The polyacrylamide gels (7%) were prepared as shown below:

Ingredient	Amount per gel
Acrylamide monomer	12.65 g
N_1N methylene-bisacrylamide	.67 g
Ammonium persulphate	.18 g
Dimethyl-amino propionitrile (DMAPN)	. 3 ml
Buffer V	190 ml

The solution was filtered, poured into a perspex mould (19 x 15 cm) and covered with a glass plate to exclude air, and allowed to polymerize in natural daylight.

Preparation of Tissue Samples

Seeds were homogenized, using a mortar and pestle in .4 ml of .1M phosphate buffer, pH 7.0 containing 1 mg/ml dithiothreitol. Young leaves were squashed in .5 ml of 50mM Hepes buffer, pH 7.5. Crude extracts were absorbed onto Whatman 3 mm chromatography paper wicks (4 x 4 mm). For acrylamide gels extracts were absorbed in thick Beckman No. 319329 paper wicks (4 x 5 mm). Each sample extract could be absorbed onto at least 4 wicks, so that each sample could be scored for 8 different enzyme systems. A slit (origin) was cut across the starch gels 5 cm from one end and wicks inserted in it. For acrylamide gels holes were punched across the gel 5 cm from one end. Up to 30 samples could be loaded into 1 gel.

Electrophoresis and Staining Methods

Electrophoresis was carried out in a cold room maintained at 4°C. The corresponding tank buffers for the gel buffers are shown in Appendix B. A 1% solution of bromophenol blue was used to track the front in starch systems 2 and 3. The borate front in starch gels of Type 1 and the front in acrylamide gels are visible without the use of any marker dye. Acrylamide gels were run at 150 volts for 4-5 hours until the front had run 8 cm from the origin. Type 1 starch gels were run at 260 V up to a maximum of 60 m amp per gel for 5-6 hours until the front had migrated 8-9 cm from the origin. Histidine starch gels were run for 6-7 hours at a constant 25 m amps per gel.

On completion of electrophoresis, and after removal of the wicks, each starch gel was cut horizontally into 2 slices, which were then stained for 2 different enzymes. Acrylamide gels could be cut into 3 slices when required. The esterases, total protein, peroxidases and acid phosphatases were best resolved on acrylamide whereas Type 1 starch gels gave good staining of alcohol dehydrogenases, glutamate dehydrogenases, leucine aminopeptidases and glutamic-oxaloacetic transaminases. Histidine gels were used to score malate dehydrogenases, although they also gave reasonable resolution of acid phosphatases and alcohol dehydrogenases. Glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase were satisfactorily resolved on Type 3 starch gels. The components of the enzyme staining solutions are given in Appendix C. These recipes were obtained and modified where required, from the methods of Brewbaker *et al* [1968], Shaw and Prasad [1970], and Brewer and Sing [1970]. Leucine aminopeptidases, glutamate-oxaloacetic transaminases and peroxidases were stained at room temperature, but for all the other enzymes optimum activity was obtained by incubating gels at 37°C in the dark.

Normally gels were scored immediately after bands reached optimum visibility. For the EST-1 and EST-2 loci this was after 5 minutes staining, but for the EST-4 locus overnight incubation was needed. Gels were fixed by placing in a wash solution (methanol:acetic acid: water, 5:1:5) for 2 hours and could be stored indefinitely wrapped in Gladwrap at 4°C. Transaminases had to be scored immediately, since they could not be satisfactorily fixed.

Enzyme nomenclature and classification

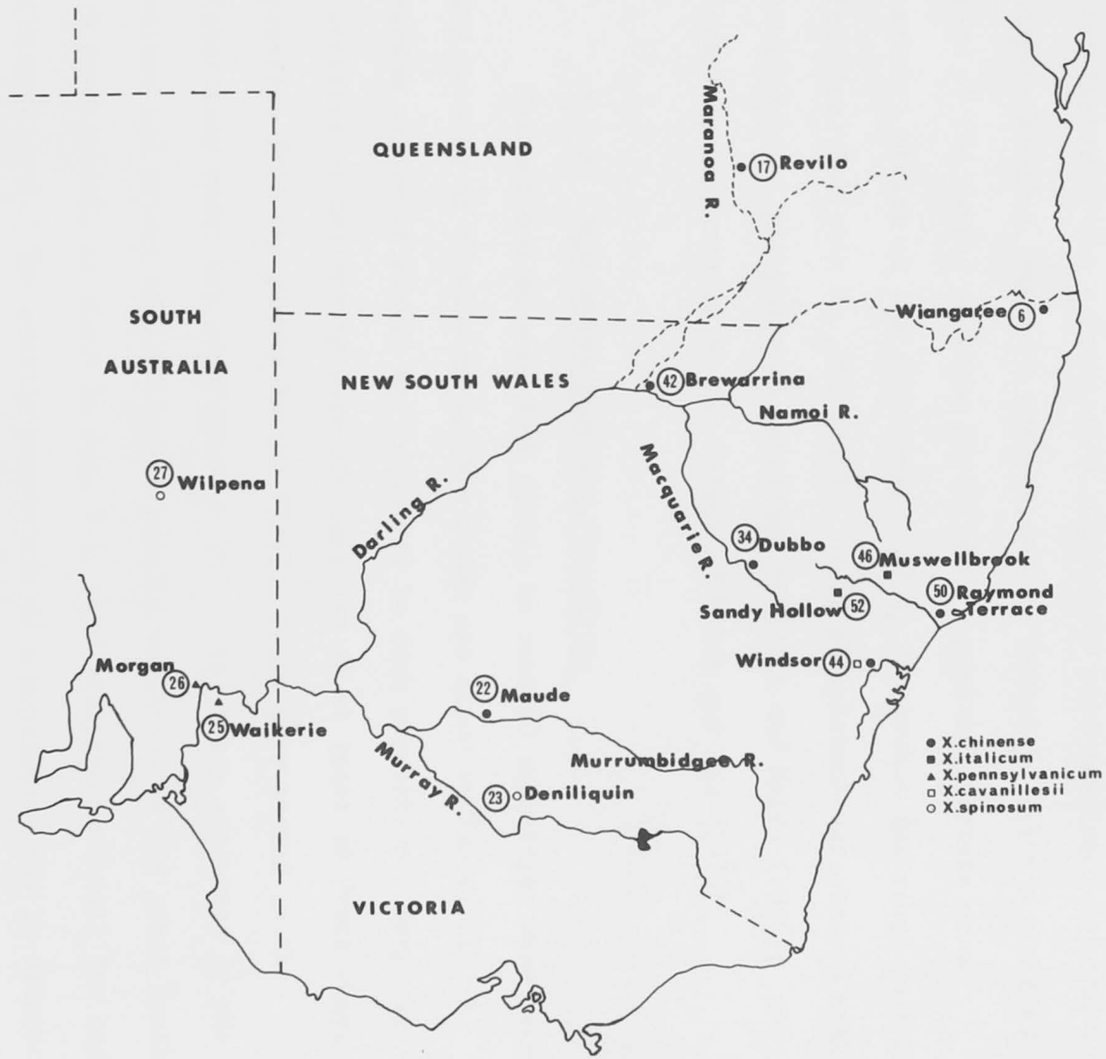
On each zymogram the different band positions were assigned numbers with the most anodal band being number 1 in all systems, as recommended by the IUPAC-IUB Commission on Biochemical Nomenclature [Anonymous, 1972]. However, bands which were not regularly scorable, or only appeared after prolonged incubation were not included in the present analysis. Unless specifically stated, the bands described on the zymograms are only those of the seed enzymes. The zymograms for the enzymes of the 2 species are considered separately, though it is clear they have very similar patterns. Within *X. strumarium*, the races are considered together such that for 1 locus the N (normal) band represents the allele occurring most frequently among the races, while other allelic bands are labelled S (slow) or F (fast) depending on their position in the gel relative to the normal band. Zones of enzyme activity on each zymogram were assigned mobility values determined by their migration distances relative to a standard band which was denoted by an electrophoretic mobility of 1.00.

The genetic basis for these isozymes was determined from the segregation in half-sib families in natural populations and an analysis of F_1 families between races. F_2 progeny have been obtained, but the analysis of these had not been completed at the time of writing.

Quantitative enzyme activity

A preliminary comparative quantitative analysis of enzyme activity in seed and young leaf tissue was carried out. For each sample 1 g fresh weight of tissue was ground into an acetone powder and then the enzymes were extracted in the extraction medium, which consisted of 50 mM Hepes KOH, pH 7.5, 4 mDTT, 5 mM $MnCl_2$ and 25 mg polyvinyl-pyrrolidone (M.W. 100,000). The protein was precipitated using 60% NH_4SO_4 solution, centrifuged down, then washed in 60% NH_4SO_4 and stored

Fig. 3.1. Map showing location of population sites analysed for allozyme variation.


QUEENSLAND
Maranoa R.
17 Revilo
SOUTH
AUSTRALIA
NEW SOUTH WALES
Wiangaree 6
42 Brewarrina
Namoi R.
27 Wilpena
Darling R.
Macquarie R.
34 Dubbo
46 Muswellbrook
50 Raymond Terrace
Sandy Hollow 52
Windsor 44
Morgan 26
22 Maude
25 Waikerie
Murray R.
Murrumbidgee R.
23 Deniliquin
VICTORIA
X.chinense
X.italicum
X.pennsylvanicum
X.cavanillesii
X.spinosum

at 4°C in this form. Immediately prior to assaying for the enzymes, the precipitate was redissolved in the extraction medium, and enzyme activities measured on these solutions. Protein determinations were done by the Lowry method [Lowry *et al*, 1951]. Both seed and young leaf tissue of the 4 races of *X. strumarium* were examined. Of the 4 enzymes studied, esterase activities were estimated using a modified form of the method of Koehn [1970] with α-naphthyl acetate or α-naphthyl oleate as the substrate; LAP by the method described in Sigma Technical Bulletin No. 251 [1974]; acid phosphatase was assayed with p-nitrophenol phosphate as described by Slack and Hatch [1967]; and transaminases assayed by the method of Hatch and Mau [1973].

3.2.2. Population Sampling Procedures

Collections of seed from plants in over 60 localities were made and details of those used in this study are shown in Table 2.1. A random sample of plants was collected in each population. Sampling consisted of collecting half-sib families of at least 10 fruit where possible from the plants in the field. Fruit were stored in the laboratory under dry conditions at 20°C. To obtain estimates of the nature and extent of qualitative genetic variation in the genus *Xanthium*, 15 populations, as shown in Fig. 3.1, were analysed in detail for seed allozyme loci. The genetic parameters of a population can be determined by analysing the progeny from individual plants in the field. In terms of resources both of time and finance, it was completely impractical to analyse the populations by the so-called "standard procedure" in which all the progeny from all the plants are scored. Hence partial progeny testing was used. Brown [1975] compared the statistical efficiency of 4 experimental designs, in which partial progeny testing was employed.

In the present study his Design IV was used. This entailed an initial assay of 2 seeds per family and if one or both are heterozygous, another sample is taken from the family to ascertain the maternal genotype to 95% certainty. Here, when a second sample had to be analysed, usually all the progeny of the half-sib family were analysed. With the benefit of hindsight, it is likely that Design II in which the initial assay was of 1 seed only would have had similar efficiency to the one employed.

Initially the 2 seeds for the first sample from the half-sib family were chosen from different fruit, but this was not pursued once it became clear from the results that it was not going to underestimate the genetic variation at all. Seeds of *X. spinosum* were extremely difficult to remove intact from the fruit so the fruit were cut longitudinally, and parts of the seed removed with a fine needle and forceps.

3.3. RESULTS

Genetic basis of isozyme variation

The discovery that much of the isozyme variation within organisms has a genetic basis has led to the term allozyme being employed to describe different enzyme forms produced by different alleles at the same locus.

There are no published reports of the genetics of isozyme variants in *Xanthium*, but there are 2 reports describing isoenzyme variation in leaves during development. Nitsan [1962] using paper and free-boundary electrophoresis examined variation in total protein patterns during floral induction but individual bands were not identified. Chen, Towill and Loewenberg [1970] looked at the isoenzyme patterns of individual leaves of *X. strumarium* with disc acrylamide

Fig. 3.2. (a) Photograph of a gel showing leucine aminopeptidase phenotypes

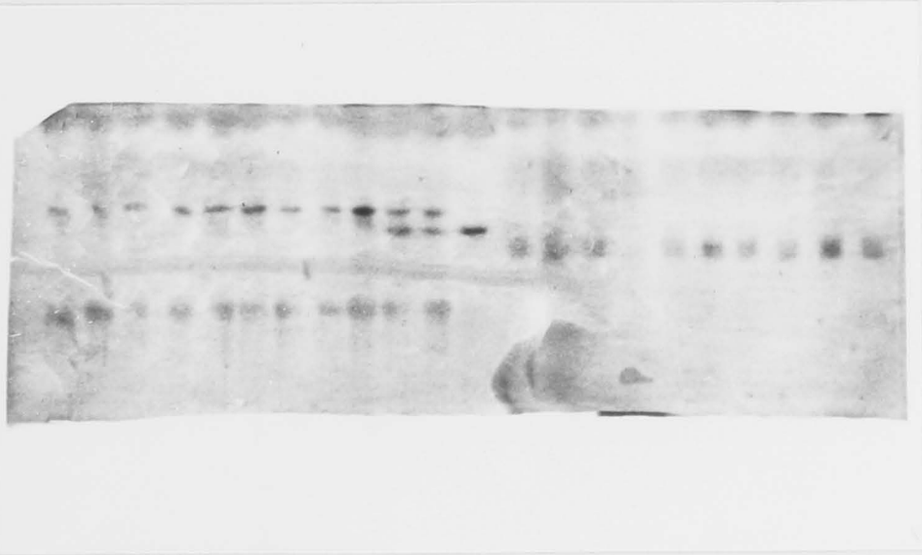
(b) Diagram representing the observed variants at LAP-1

(1) $LAP\text{-}1^{NN}$

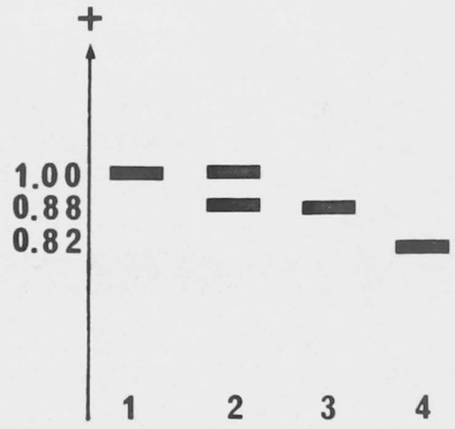
(2) $LAP\text{-}1^{NS}$ - *X. strumarium*

(3) $LAP\text{-}1^{SS}$

(4) LAP-1 - *X. spinosum*



(a)



(b)

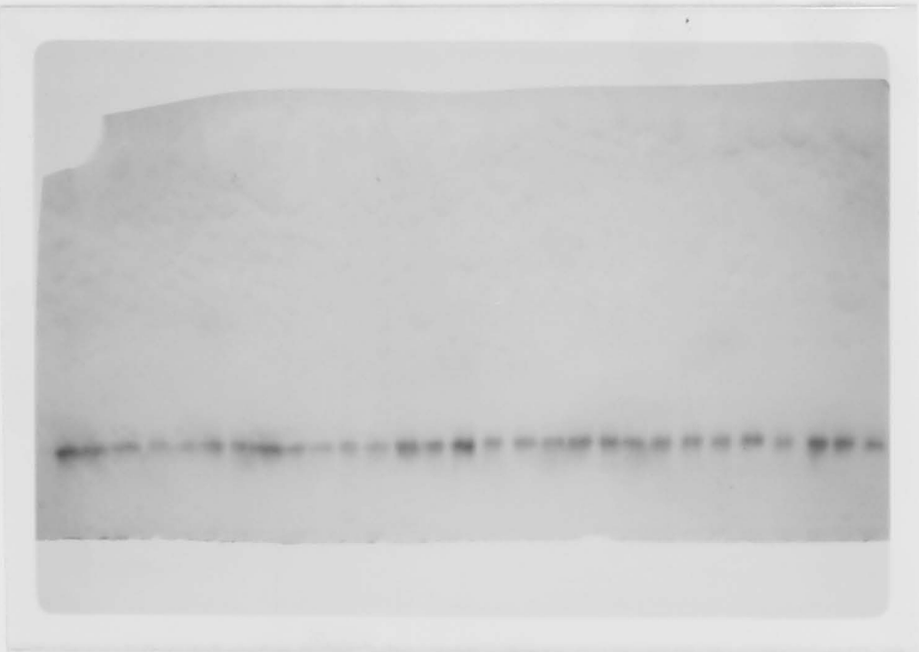
Fig. 3.2

Fig. 3.3. (a) Photograph of a gel showing GDH-1 phenotype of *X. strumarium*

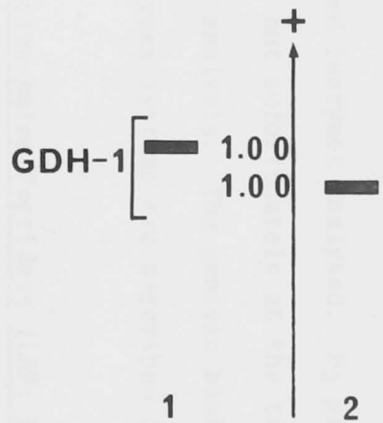
(b) Diagram representing the observed variants in

(1) GDH-1^{NN} - *X. spinosum*

(2) GDH-1^{NN} - *X. strumarium*



(a)



(b)

Fig. 3.3

electrophoresis; but no genetic interpretation was made of the bands. Isozymes of seeds of *Xanthium* have not been previously reported.

Crosses between races of *X. strumarium* were made and the isozyme patterns of F_1 seed isozymes analysed. F_2 progenies are in the process of being obtained but unfortunately at the time of writing they were not yet ready for analysis. The genetic basis of electrophoretic variants of 8 enzymes systems are described below.

3.3.1. Leucine Amino Peptidase (LAP: EC 3.4.1.1.)

Enzyme activity is observed on starch gels (Type 1) at 1 zone, which corresponds to 1 locus. There are 2 variants at this locus in *X. strumarium* but only 1 allele in *X. spinosum* and their electrophoretic mobilities are shown in Fig. 3.2. The enzyme appears to be a monomer since the heterozygotes at this locus have only the parental bands. Crosses between the 2 different homozygotes gave F_1 seed which have both parental bands. It has been shown in other plants, such as maize [Beckman *et al*, 1964] and *Arabidopsis* [Grover, 1974], that the leucine amino-peptidases are monomers. This locus is not detectable in roots or leaves of either species.

3.3.2. Glutamate Dehydrogenase (GDH; EC 1.4.1.2.)

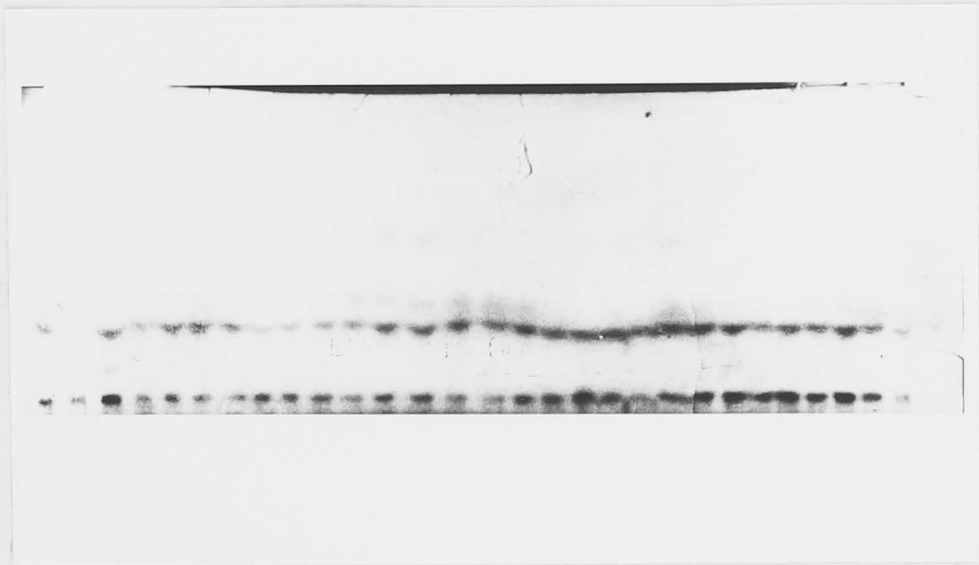
Glutamate dehydrogenase typically stains blue on starch gels (Type 1) with the use of NBT. There was only 1 site of activity detectable in seeds, and none in other tissues. In each species there is a single distinct invariant band but these have different mobilities as shown in Fig. 3.3.

Fig. 3.4. (a) Photograph of a gel showing malate dehydrogenase phenotypes in *X. strumarium*

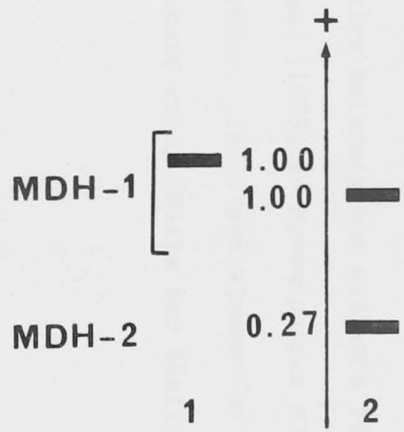
(b) Diagram representing observed variants at MDH loci

(1) MDH-1^{NN} - *X. spinosum*

(2) MDH-1^{NN}/MDH-2^{NN} - *X. strumarium*



(a)



(b)

Fig. 3.4

Each band has been interpreted as a single allele at 1 locus. None of the complex heterozygous patterns reported by Pryor [1974] in maize or by Babbel [1975] in *Hymenopappus scabiosaeus* have been observed in *Xanthium*. In *Datura stramonium* [Carlson, 1972] and species of the wheat group [Mitra and Bhatia, 1971] no GDH variants were found.

3.3.3. Malate Dehydrogenase (MDH; EC 1.1.1.37)

Zones of activity are detectable on only histidine starch gels after overnight incubation. There are 2 main zones of activity with 2 other fainter more anodal bands in *X. strumarium*. The latter come up after overstaining of the gel and have not been included in the zymogram shown in Fig. 3.4. Also under conditions of prolonged staining the ADH main band of some races of *X. strumarium* can be detected with an intermediate mobility between the 2 MDH loci. The 2 MDH loci were not detectable in other tissues, but another zone was present in young leaves, which could be the same as the intense band of young leaves described by Chen *et al* [1970]. Brewer *et al* [1969] reported 2 invariant bands in seeds of 38 wheat strains, while Mitra and Bhatia [1971] found no isoenzyme variaton in MDH of dry seeds from several species of the subtribe *Triticinae*. Hubby and Lewontin [1966] showed MDH in *D. pseudoobscura* to be a dimer but there are few published genetic analyses of MDH isozymes in plants [Longo and Scandalios, 1969; Brown *et al*, 1974].

Fig. 3.5. (a) Diagram showing GOT loci and alleles in

(1) *X. spinosum*

(2) *X. strumarium*

(b) Photograph of a gel showing the 3 GOT-3 phenotypes in *X. italicum*

(c) Photograph of a gel showing parental and F_1 phenotypes at GOT-3 locus.

(1) GOT-3^{NN} *(X. chinense)*

(2) GOT-3^{SS} *(X. cavanillesii)*

(3) GOT-3^{NS} (F_1 *X. chinense* x *X. cavanillesii*)

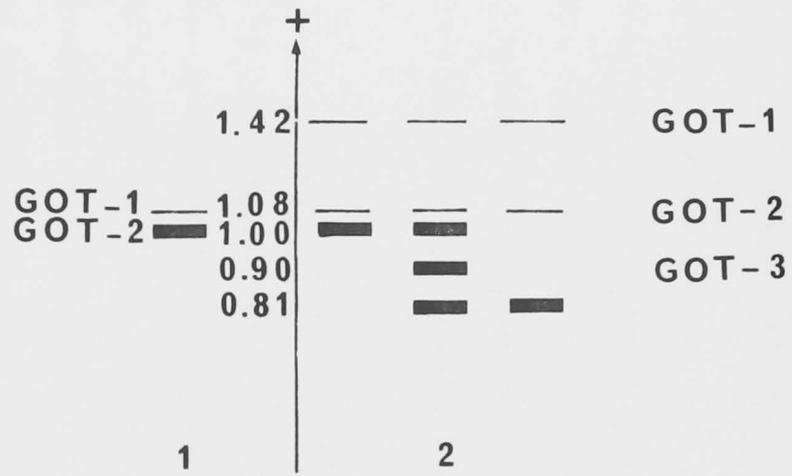
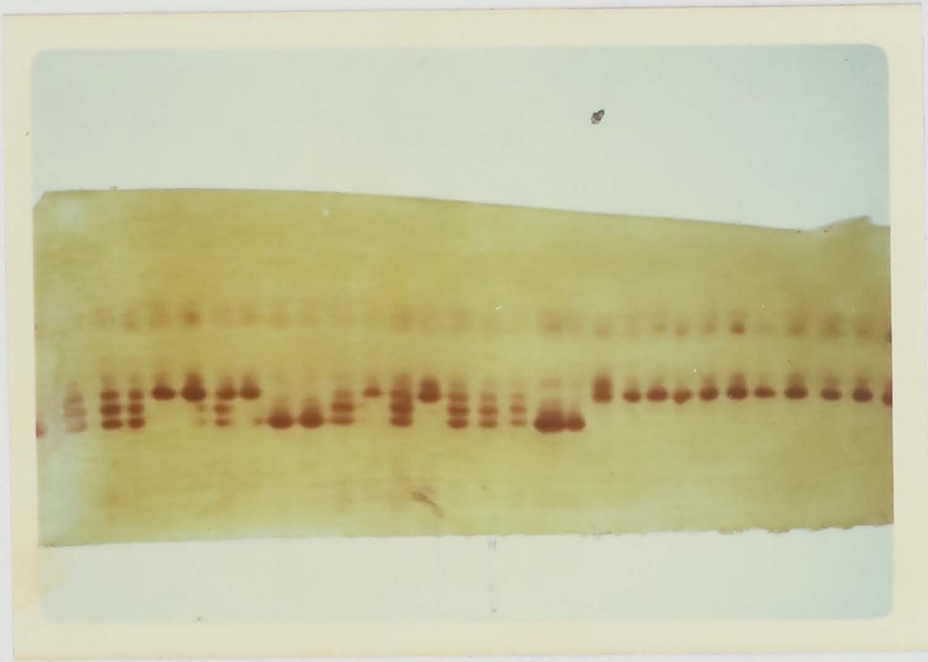
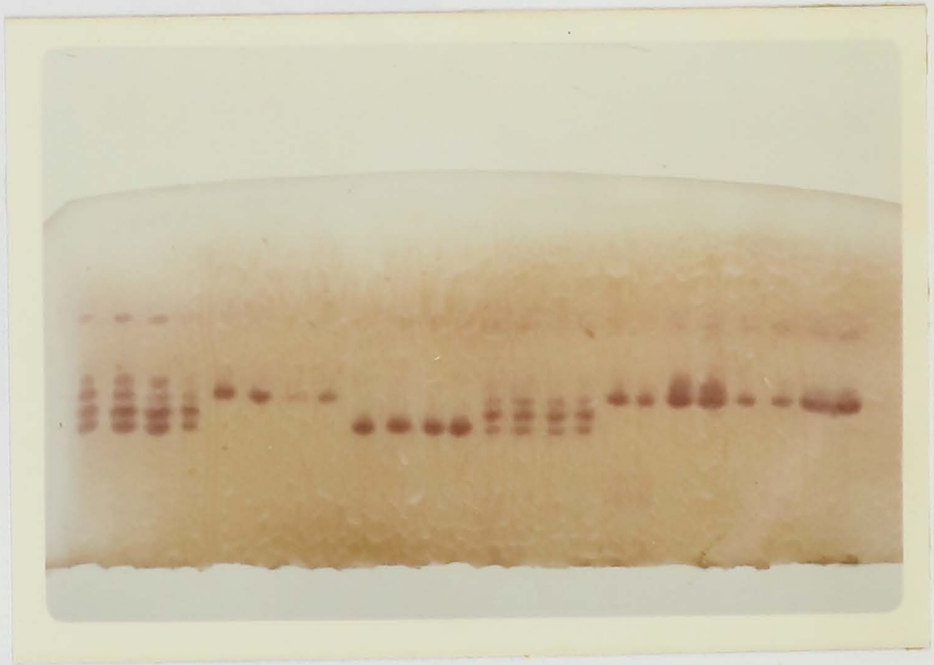


Fig.3.5(a)





1 2 3

(b) Fig. 3.5 (c)

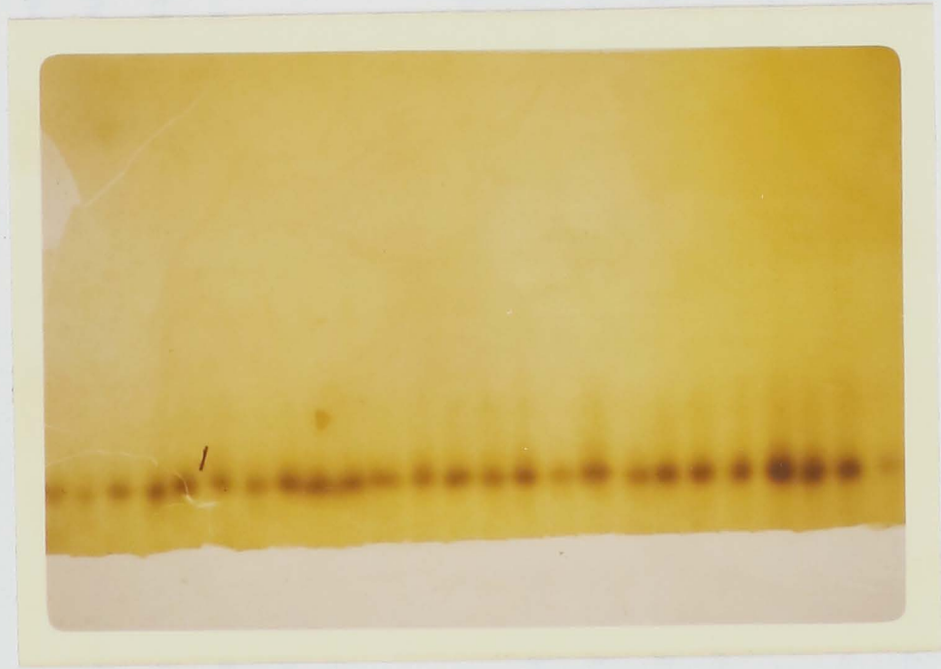


Fig. 3.6. Photograph of a gel showing acid phosphatase phenotype in *X. spinosum* and *X. strumarium*.

3.3.4. Glutamate Oxaloacetate Transaminases (GOT: EC 2.6.1.1.)

Enzyme bands are typically red coloured and 3 different alleles have been detected in *X. strumarium*. A slow variant has been detected at GOT-3 and the electrophoretic mobilities are shown in Fig. 3.5. No electrophoretic variants at GOT-1 and GOT-2 have been found. GOT-1 and GOT-3 were detectable only in seeds, whereas GOT-2 was also in cotyledons and young leaf tissue. Heterozygotes have a hybrid band suggesting that the protein is a dimer. This is also the case in maize [MacDonald and Brewbaker, 1972], *Stephanomeria* [Gottlieb, 1973a] and other organisms [De Lorenzo and Ruddle, 1970]. Only 2 loci were detectable in *X. spinosum*. In *X. strumarium* the F_1 seeds from crosses of the 2 homozygotes all had the 2 parental bands and a hybrid band of intermediate mobility at GOT-3.

3.3.5. Acid Phosphatases (AcPh; 3.1.3.2.)

Phosphatase activity could be detected on Type 2 starch gels, or acrylamide after 3 hours incubation. In seeds, only 1 zone of activity was detectable (Fig. 3.6). This consisted of a single band identical in *X. spinosum* and *X. strumarium*. A separate more anodal locus is present in young leaves of *X. strumarium*. This is not included in the present classification since very few plants were screened for variants at this locus.

3.3.6. Esterases

Several zones of esterase activity have been observed on acrylamide gels. The seed isozymes are shown in Fig. 3.6, but the leaf esterase isozymes are markedly different, and have not been analysed. In seeds, overstaining produced other bands most of which are more anodal than EST-1. These have not been included in the present analysis. No variants have been detected at EST-1, EST-2 or

Fig. 3.7. (a) Diagram showing observed variants at EST-4

(1) EST-4^{NF}

(2) EST-4^{SS}

(3) EST-4^{NN}

(b) Photograph of a gel showing EST-1 and EST-2 phenotypes

(c) Photograph of a gel showing phenotypes at EST-4

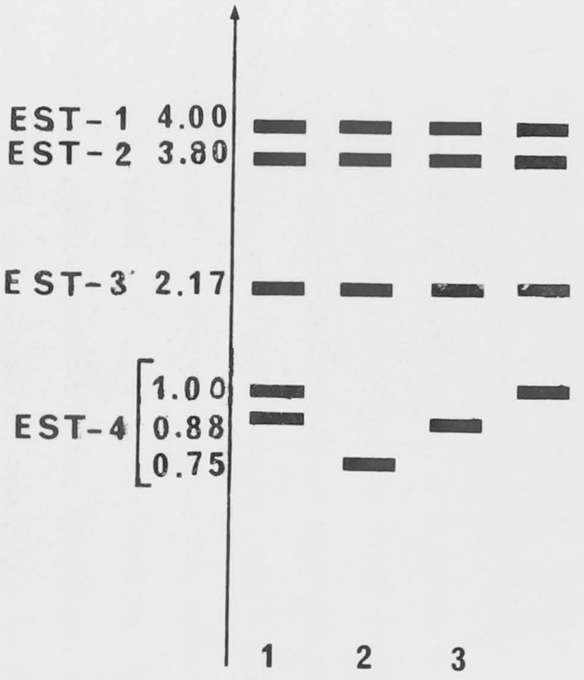
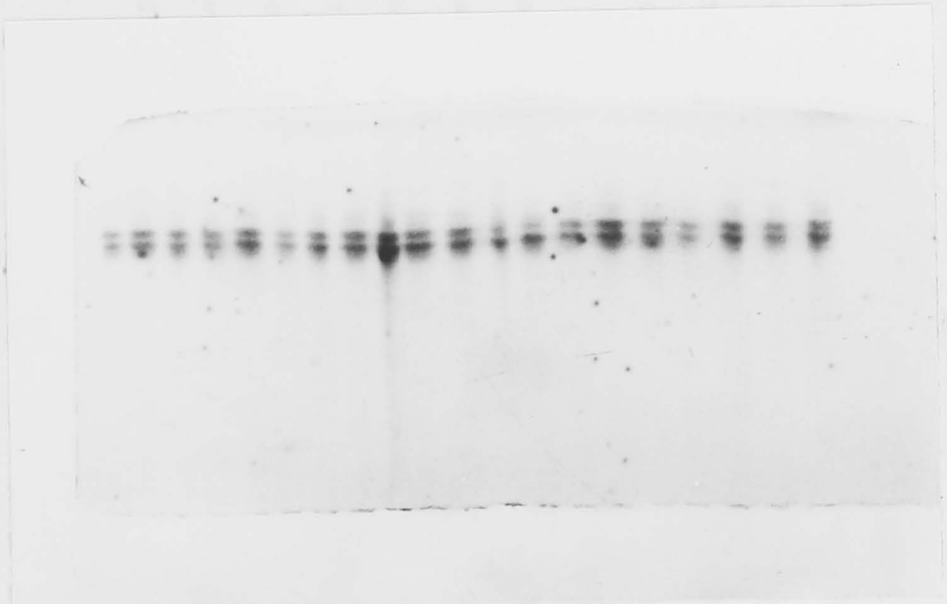
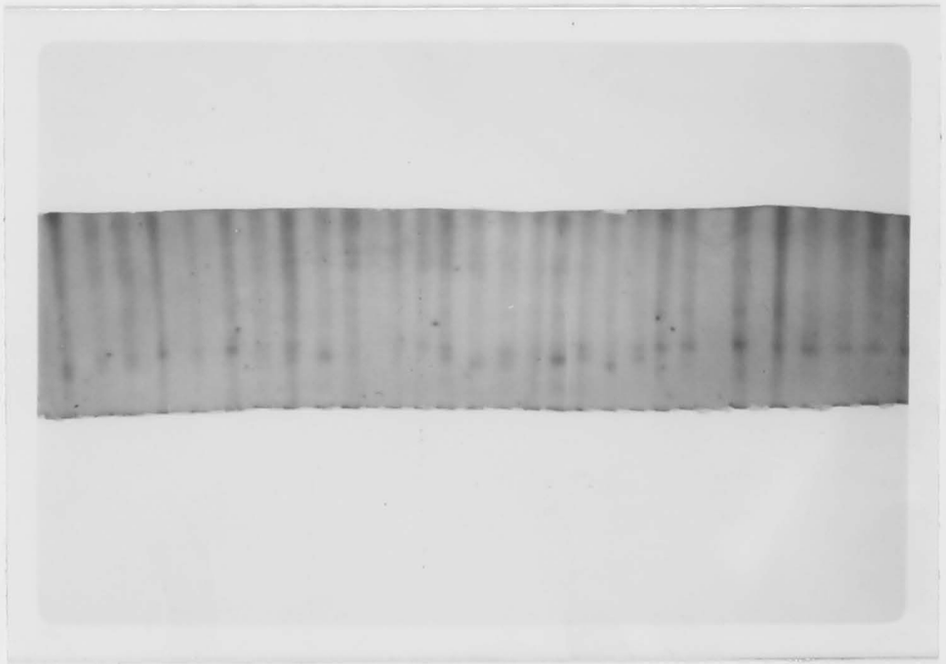


Fig. 3.7(a)



(b)

Fig. 3.7



(c)

EST-3. There are 3 alleles at EST-4, however, and from crosses between different homozygotes the F_1 seed have the 2 parental bands without hybrid band formation. The genetics of esterases of several plant species have been analysed [Scandalios, 1974]. Some, such as those in *Avena* [Marshall and Allard, 1969] and *Arabidopsis* [Grover, 1974] appear to be monomers, while for maize some of the esterases are dimers and other monomers [MacDonald and Brewbaker, 1972; Brown and Allard, 1969]. The esterase zymograms of developing *Xanthium* leaves, described by Chen *et al* [1970], do not seem to have the same bands as seeds. In *X. spinosum* several closely spaced bands were present, but these could not be resolved satisfactorily and have not been studied further.

3.3.7. Alcohol Dehydrogenase (ADH; ED 1.1.1.1.)

Alcohol dehydrogenase could be detected in any of the starch gel systems, but Type 1 gels gave the best resolution (Fig. 3.8). The isozyme patterns are somewhat unusual compared to those found in other plant species. In *X. spinosum* the single zone of activity has been interpreted as a single locus, and no variants have been detected at this locus. In *X. strumarium* the bands have been interpreted as 2 loci with 2 alleles at ADH-2 and 1 allele at ADH-1. The normal N (1.00) band at the ADH-2 locus is very often twice as intense as the other 2 bands. It is suspected therefore that this band is in fact 2 duplicated loci and that the other 2-banded patterns arose by mutations from the N allele at the original ADH-1 locus. Duplicate ADH genes have been reported in wheat [Hart, 1970] and *Clarkia franciscana* [Gottlieb, 1974]. For nearly all plant species which have been studied, at least 2 ADH

Fig. 3.8. (a) Diagram representing the observed variants at ADH-1 and ADH-2 in

(1) *X. spinosum*

(2) *X. strumarium*

(b) Photograph of a gel showing ADH phenotypes in

(1) *X. strumarium*

(2) *X. spinosum*

(c) Photograph of a gel showing ADH phenotypes in

(1) *X. chinense*

(2) *X. pennsylvanicum*

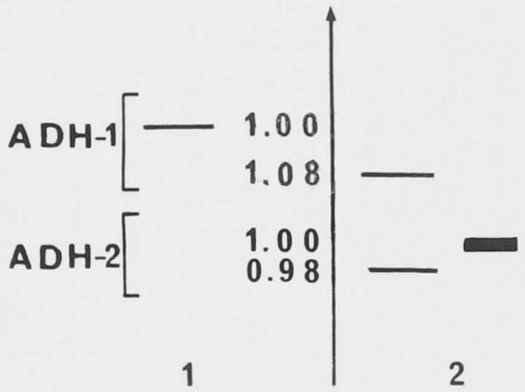
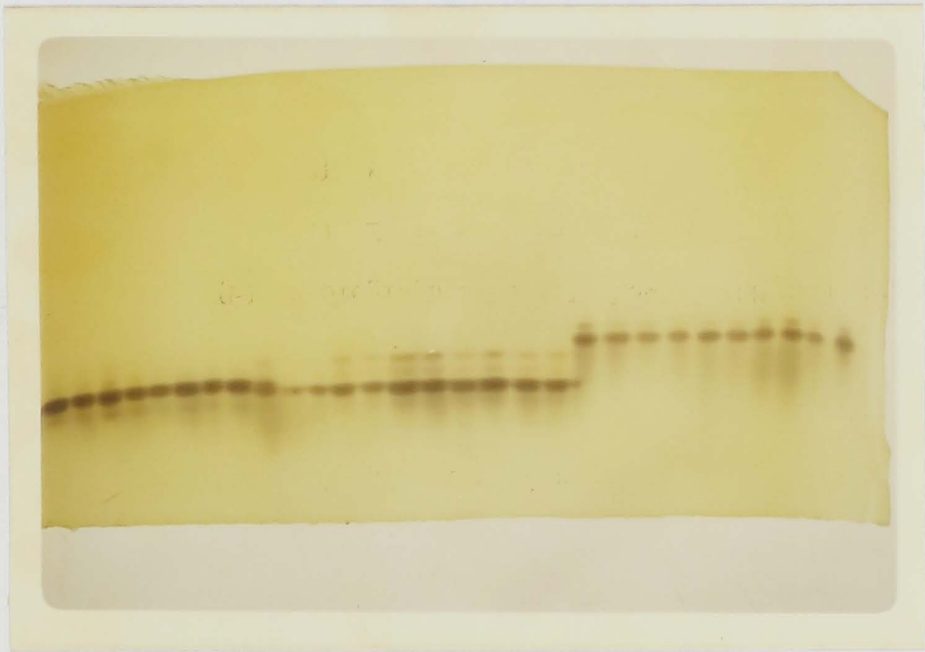


Fig. 3.8(a)



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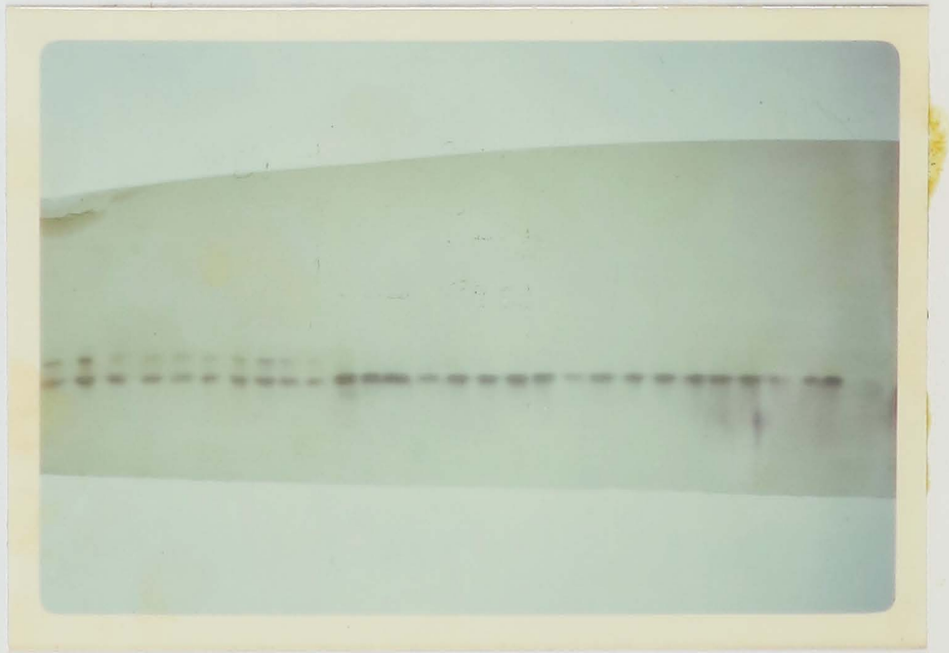
(b)



1 2

(c)

Fig.3.8

loci have been detected including sunflowers [Torres, 1974], maize [Scandalios, 1974; Freeling, 1974]; narrow-leafed lupins [Marshall *et al*, 1974] safflower thistles [Efron *et al*, 1973] and eucalypts [Brown *et al*, 1975]. If plants with the 2 different banding patterns are run as 1 sample the 2 ADH-2 alleles cannot be resolved satisfactorily for the much more intense N allele makes the S allele indistinguishable. F_1 seed from crosses between these 2 different banding patterns have an intense band in the N allele region of ADH-2 and nearly always the ADH-1 allele. Alcohol dehydrogenase activity has not been detected in normal or waterlogged roots or green tissue. However, all bands are present in the endosperm as well as the embryo.

3.3.8. Glucose-6-Phosphate Dehydrogenase (G6PD; EC 1.1.1.49)

Both glucose-6-phosphate dehydrogenase (G6PD) and 6-phosphogluconate dehydrogenase (6-PGD) could be successfully resolved on Type 3 starch gels. Only G6PD has been studied. Both species have the same zone of activity. Very few individuals of *X. strumarium* have been screened for this enzyme. There is a single band, identical in both species, and assumed to be a single locus with 1 allele. With prolonged staining ADH activity can be detected when assaying for G6PD, but the G6PD band is faster migrating than the ADH bands. Very few if any genetic analyses of G6PD isozymes have been published although G6PD leaf isozymes have been used in plant population analyses [Levin, 1975]. Sing and Brewer, [1969] reported G6PD isozymes in wheat seeds.

TABLE 3.1.

ENZYME ACTIVITY* IN SEEDS OF THE 4 RACES OF *X. STRUMARIUM*

	X. chinense		*X. italicum*		*X. pennsylvanicum*		*X. cavanillesii*	
	mg protein^{-1}	g D wt^{-1}	mg protein^{-1}	g D wt^{-1}	mg protein^{-1}	g D wt^{-1}	mg protein^{-1}	g D wt^{-1}
Esterase (x 10^{-2})								
- acetate	18.0	53.3	10.8	54.3	17.4	38.0	23.6	73.5
- oleate	3.3	9.7	14.2	42.0	5.4	11.7	1.3	4.0
Leucine amino peptidase	1.59	5.37	2.13	8.62	1.55	3.71	3.07	9.72
Acid phosphatase	10.38	35.11	4.89	19.62	4.67	11.14	6.73	21.50
Aspartate amino transferase (GOT)	0.52	1.75	0.81	3.28	0.37	0.89	0.55	1.76

* Δ O.D. min^{-1}

3.3.9. Other Systems

No enzyme activity was observed in seed or leaf tissue when gels were tested for amylases, catalases, alkaline phosphatases and several other dehydrogenases. In seed, no definite peroxidase bands were detected but 2 zones of activity were resolved from leaf tissue. There were 2 bands in *X. strumarium* but no variants were seen and this was confirmed by the recent work of McMillan [1975c]. Zones of tetrazolium oxidase acitivty were observed on starch gels in seed samples of both species, but were not regularly scorable. At least 5 separate zones of total protein activity were detectable on acrylamide gels in seed samples of *X. strumarium* , but difficulties with background stain has so far prevented further study of these isozymes. Total protein bands could not be resolved satisfactorily from seeds of *X. spinosum*.

Attempts to reproduce the results of Chen *et al* [1970] with leaves using either disc acrylamide electrophoresis or our standard horizontal electrophoresis procedures were largely unsuccessful.

3.3.10 Quantitative Enzyme Activity

The results of a preliminary study of the activity of 4 enzymes in seed and young leaf (6 days old) tissue for the 4 races of *X. strumarium* are shown in Table 3.1. Enzymes have not been purified, so that assays are on fairly crude extracts. The rationale of the experiment is that with different alleles fixed in different races then for a particular enzyme significant differences in activity between certain races will be due to functional differences between the alleles. Only 1 set of experimental conditions was used; namely room temperature (22°C) and the accepted pH optimum of the enzymes. *X. cavanillesii* is

fixed for the S allele at GOT-3 while the other 3 races have the N allele. The activity for this enzyme in this race is not significantly different to that for the other races. There is only 1 LAP locus in seeds, yet the S allele in *X. pennsylvanicum* has similar activity to the N allele in the other 3 races.

Similarly, at the EST-4 locus the S allele of *X. pennsylvanicum* has similar activity to the F allele of *X. chinense*. The low figure for *X. italicum* could be an indication of low activity of the N allele at EST-4, but the composition of the *X. italicum* assay samples in terms of N and S alleles was not determined. However, the esterase estimations are the least accurate of the enzyme determination. Further work is required to see if alleles have the same properties over a range of temperature and pH values. For these 4 enzymes no significant differences in activity between alleles could be detected.

3.4. GENETIC ANALYSIS OF POPULATIONS

(a) *X. strumarium*

In all, 12 populations of *X. strumarium* were analysed and the geographic position of these, except population 11, are shown in Fig. 3.1. Seven enzyme systems, described by 13 enzyme loci, were assayed. The allelic frequencies at these loci are shown in Table 3.2. In this Table, dashes indicate that the allele or locus was not detectable in the particular population, whereas blank spaces indicate that the population was not scored for that locus.

The most striking feature of this data is the lack of allozyme variability within the races of the species. Moreover there is a complete lack of intrapopulation variation, except at 2 loci in 2 populations of *X. italicum*. For the other 3 races, the data suggest that all individuals within a race are homozygous for the same alleles

TABLE 3.2.

GENE FREQUENCIES AT LOCI IN *X. STRUMARIUM*

Site	Locus alleles		Est-1	Est-2	Est-4			AcPh-1	GDH-1	MOH-1
			N	N	F	N	S	N	N	N
		N				*X. pennsylvanicum*				
25	Waikerie	66	1.0	1.0	-	-	1.0	1.0	1.0	1.0
26	Morgan	128	1.0	1.0	-	-	1.0	1.0	1.0	1.0
						X. italicum				
46	Muswellbrook	146	1.0	1.0	.85	.13	.02		1.0	1.0
52	Sandy Hollow	78	1.0	1.0	.79	.21	-		1.0	
						X. chinense				
6	Wiangaree	59	1.0	1.0	-	-		1.0	1.0	
11	Oxford	168	1.0	1.0	1.0	-	-	1.0	1.0	
17	Revilo	124	1.0	1.0	1.0	-	-		1.0	1.0
22	Maude	126	1.0	1.0	1.0	-	-	1.0	1.0	
34	Dubbo	177	1.0	1.0	1.0	-	-	1.0	1.0	
42	Brewarrina	176	1.0	1.0	1.0	-	-	1.0	1.0	
50	Raymond Terrace	115	1.0	1.0					1.0	
						X. cavanillesii				
44	Windsor		1.0	1.0	-	-	- *	1.0	1.0	1.0

Est-4 could not be detected in *X. cavanillesii*

TABLE 3.2. (cont.)

GENE FREQUENCIES AT LOCI IN *X. STRUMARIUM*

Site	Locus alleles		MDH-2	ADH-1	ADH-2		LAP 1		GOT-1	GOT-2	GOT-3	
		N	N	N	N	S	N	S	N	N	N	S
						X. pennsylvanicum						
25	Waikerie	66	1.0	-	1.0	-	-	1.0	1.0	1.0	1.0	-
26	Morgan	128	1.0	-	1.0	-	-	1.0	1.0	1.0	1.0	-
						X. italicum						
46	Muswellbrook	146	1.0	-	1.0	-	1.0	-	1.0	1.0	.98	.02
52	Sandy Hollow	78		-	1.0	-			1.0	1.0	1.00	-
						X. chinense						
6	Wiangaree	59		1.0	-	1.0	1.0	-	1.0	1.0	1.0	-
11	Oxford Downs	168		1.0	-	1.0	1.0	-	1.0	1.0	1.0	-
17	Revilo	124	1.0	1.0	-	1.0	1.0	-	1.0	1.0	1.0	-
22	Maude	126		1.0	-	1.0	1.0	-	1.0	1.0	1.0	-
34	Dubbo	177		1.0	-	1.0	1.0	-	1.0	1.0	1.0	-
42	Brewarrina	176		1.0	-	1.0	1.0	-	1.0	1.0	1.0	-
50	Raymond Terrace	115					1.0	-	1.0	1.0	1.0	-
						X. cavanillesii						
44	Windsor	62	1.0	-	1.0	-	1.0	-	1.0	1.0	-	1.0

at all 13 loci. In fact, the results could be interpreted as showing that, within Australia, the populations of each race represent a single monogenic strain.

While there could be variation at other loci not assayed, these data demonstrate that the levels of variability are very low within each race. However, there are clear genetic differences between races. Some loci are fixed for the same allele in all races, while at other loci (e.g. EST-4, LAP-1, GOT-3, ADH-2) some races are fixed for the same allele, and others for alternate alleles. For example, in *X. pennsylvanicum* all plants are homozygous for the S allele, at the LAP-1 locus while the other 3 races are fixed for the N allele. Similarly, *X. cavanillesii* is fixed for the S allele at GOT-3, while the other races have plants homozygous for the N allele (except for population 46). It is noticeable that *X. chinense*, which has by far the widest geographical distribution (see Fig. 2.3) is represented by 1 genotype at the 13 loci. It is also clear that some loci are very good diagnostic loci [as defined by Lewontin, 1974] for certain races. Thus detection of an S allele at the LAP-1 locus in a seed would most certainly mean that the seed belonged to the *X. pennsylvanicum* race.

Considering all the races as part of 1 species, then the proportion of loci which are polymorphic for *X. strumarium* as a whole is 0.31. This is fairly meaningless however, since it was shown in Chapter 2 that, at least in Australia with the present distribution of the 4 races, only *X. italicum* and *X. chinense* could interbreed. *X. italicum* and *X. chinense* have very similar genotypes, and apart from the extra ADH locus in *X. chinense*, the main difference is the genetic segregation at 2 loci in *X. italicum*. The mean heterozygosity per locus per individual in *X. pennsylvanicum*, *X. chinense* and *X. cavanillesii* is 0. The genotypic frequencies at the EST-4 locus in *X. italicum* point

TABLE 3.3.

GENOTYPIC FREQUENCIES AND FIXATION INDICES IN 2 POPULATIONS OF *XANTHIUM ITALICUM*

Population	Locus	FF	FN	NN	NS	SS	$\hat{F}$*	F_e^{Φ}
46	EST-4	.82	.06	.10		.02	.79	.98
52	EST-4	.77	.05	.18		.00	.83	.96
46	GOT-3			.97	.02	.01	.49	.98

*$\hat{F} = 1 - \hat{R}/2\hat{q}_1(1-\hat{q}_1)$ where R = observed level of heterozygozity and $\hat{q}$ = frequency of F allele

$^{\Phi}F_e = \frac{(1 - t)}{(1 + t)}$ assuming no selection is the equilbrium value of the inbreeding coefficient.

to low numbers of heterozygotes, indicative of high inbreeding (Table 3.3.). The percentage of heterozygotes at EST-4 is 6 and 5% in populations 46 and 52 respectively.

The genotypes of the mother plants could be determined from all half-sib families without recourse to maximum likelihood estimates [Brown, 1975]. After the first sampling of the population, only families showing a heterozygote in the first survey were further analysed, and these always had 3 genotypes present. However, this means that t, the outcrossing rate, can only be estimated from the probability that the next homozygous mother parent analysed in the population will have at least 1 heterozygous offspring. This will be a maximum estimate. At both loci in population 46, $\hat{t} = .007$, and in population 52 the rate of outcrossing was $\hat{t} = .015$. Estimates of Wright's fixation index (F) are shown in Table 3.3. These can be compared to the theoretical inbreeding coefficients (F_e) to test whether selection favours heterozygotes differentially in the 2 populations. The F_e values in Table 3.3. indicate a marked excess of heterozygotes over expected values and suggest that selection favouring heterozygotes may be occurring at these loci. Ideally, rates of outcrossing should be estimated over several seasons to determine whether significant fluctuations occur in t values as this could possibly lead to higher expected frequencies of heterozygotes [Nei, 1975]. In an initial survey of *Xanthium* populations for electrophoretic variants, at least 5 mother plants from 18 populations of *X. chinense* were screened for, on the average, 7 loci. No variants were observed.

TABLE 3.4.

GENE FREQUENCIES AT LOCI IN 3 *X. SPINOSUM* POPULATIONS

		N	GDH-1	ADH-1	MDH-1	G6PD-1	LAP-1
			N	N	N	N	N
23	Deniliquin	90	1.0	1.0	1.0	1.0	1.0
27	Wilpena	60	1.0	1.0	1.0	1.0	1.0
43	Coolgardie	60	1.0	1.0	1.0	1.0	1.0

		AcPh-1	GOT-1	GOT-2
		N	N	N
23	Deniliquin	1.0	1.0	1.0
27	Wilpena	1.0	1.0	1.0
43	Coolgardie	1.0	1.0	1.0

(b) *X. spinosum*

X. spinosum has a more temperate distribution compared to that of *X. chinense*, such that the majority of *X. spinosum* populations collected were from the southern half of the continent. Partly because this study has centred on *X. strumarium* and its races, but also because of the technical difficulties of extracting seeds from burrs for analysis, only 3 populations of this species were studied in detail (Table 3.4.). These sites are very widely separated geographically and the chances of gene flow between them by the usual dispersal mechanisms of water or stock are very slight.

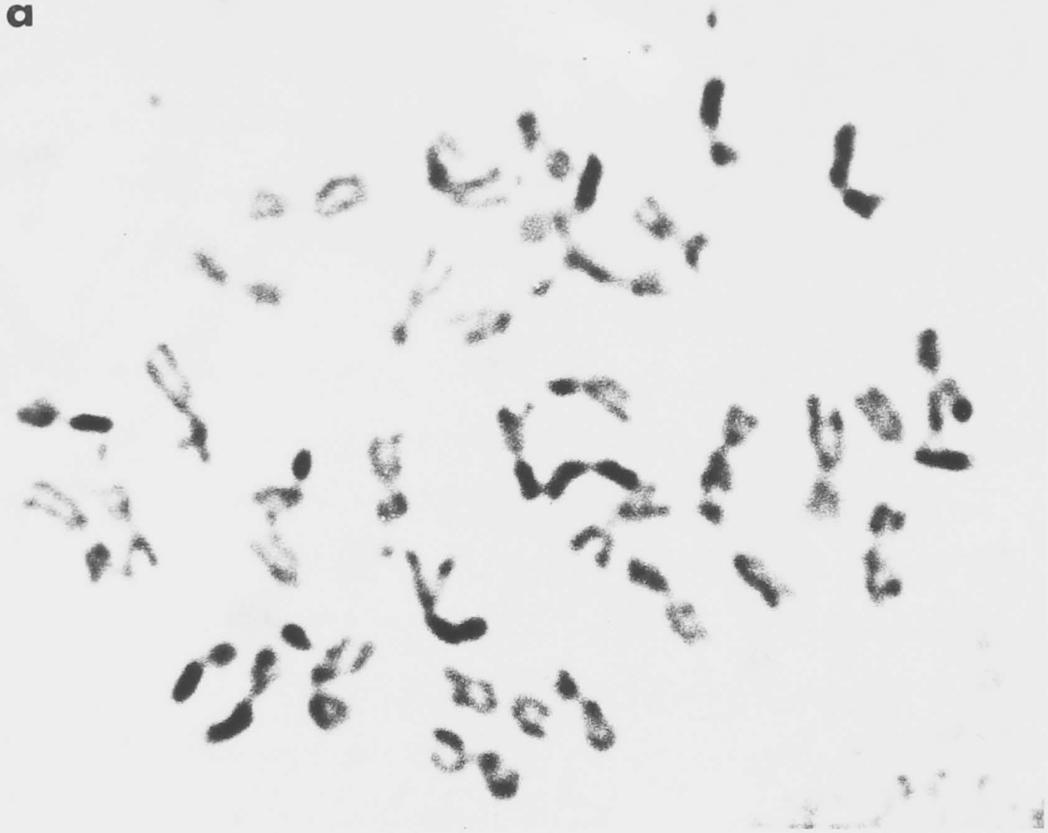
The results show that, at least for these populations, all plants are homozygous at the 8 loci and in effect have the same genotype. This is similar to the situation for the other widespread and successful colonizing species, *X. chinense*. Admittedly, the normally highly

Fig. 3.9. Mitotic chromosomes of *Xanthium* x *Ca*. 4000

(a) *X. chinense* - untreated

(b) *X. pennsylvanicum* - colchicine treated.

a



b

variable esterases have not been studied in this species, and half of the loci are dehydrogenases, so these results may be an underestimate of the genetic variation. Nevertheless, the levels of genetic variation within and between populations of *X. spinosum* must be remarkably low.

3.5. CHROMOSOMAL VARIATION

All previous reports of chromosome numbers in the genus *Xanthium* have given the chromosome number as 2n = 36 [listed in Moore, 1973; Symons, 1926]. However, very few of the complexes of *X. strumarium* have been examined, and those "species" that have been examined nearly all belong to the "strumarium" complex, based on the taxonomy of the genus used in this present study. No workers have specifically compared the chromosomes of different complexes. Payne *et al* [1964] claim that "neither polyploidy nor aneuploidy is known in the genera *Dicorea*, *Hymenoclea* or *Xanthium*." They give evidence of a polyploid series in *Ambrosia*, and suggest that all the data points to a primitive chromosome number of the subtribe Ambrosiinae as n = 18, with presumably a polyploid origin derived from non-ambrosioial composites with n = 9.

Mitosis in the primary root tips of the species was examined. Roots were fixed in 3:1 alcohol, acetic acid and stained with 1% lactic propionic orcein. Normally root tips were pretreated with .05% colchicine for 30-60 minutes. In all races and both species, it was found that chromosome counts gave 2n = 36 (Fig. 3.9.). Likewise, from pollen mother cells and pollen mitosis the basic chromosome number was found to be n = 18. Also pollen grains appeared to be binucleate as has been reported for other composites [Brewbaker 1957]. Fifty half-sib families of each of the *X. chinense* and *X. pennsylvanicum* complexes were sampled for pollen, and it was found that pollen viability was

greater than 99%, on the basis of staining with lactophenol blue. The chromosomes of the races looked very similar, with a large number of metacentrics in the chromosome complement of each race, but because of their small size and the large number of them, more precise comparisons were not made. The small size of the chromosomes has made it difficult to determine whether the species of *Xanthium* are allotetraploids or autotetraploids, though the latter is considered more probable.

Overall, at the chromosomal level, there were no obvious major differences between the races of *X. strumarium*. It was difficult to distinguish between the chromosome complements of these races and this is not altogether surprising since the races can be readily crossed. However, the chromosomes of *X. spinosum*, although not different in number, appeared longer and thinner than those of *X. strumarium*.

3.6. DISCUSSION

The study of the allozyme variation in *X. strumarium* has revealed that the existing genetic variation is almost entirely between the races. The 7 populations of *X. chinense* are monomorphic at all the loci examined. For the taxonomically uniform *X. spinosum* the allozyme data demonstrated genetic uniformity in qualitative varition comparable to that within 3 of the 4 races of *X. strumarium*. Perhaps the data for *X. spinosum* are underestimates of the genetic variation due to lack of esterases in the analyses, but several other general enzymes as well as the specific enzymes have been screened for allozyme variation. The small number of genotypes within the genus *Xanthium* is in marked contrast to that found generally in other plant species, including inbreeders [Allard and Kahler, 1972a], though there is only a single genotype over a large part of the range of *A. barbata* in California [Allard and Kahler, 1972b]. However, the predominately inbreeding

weed, *Oenothera biennis* has been shown to have 1 genotype in 59% of the populations and 2 genotypes in 27% of the populations but this species has a particular chromosomal system of permanent translocation heterozygosity. From the study of the mitotic and meiotic chromosomes of *Xanthium* species all had a 2n number of 36, with no obvious chromosome differences between races of *X. strumarium*, So, with no evidence of novel chromosomal systems, it is very unlikely that this is the source of the origin or maintenance of such few genotypes in the *Xanthium* genus. In this present study, apomixis was not observed. As well, analysis of F_1 seed allozymes indicated normal meiotic exchange of genetic material, such that for the *Xanthium* genus generally there is a normal, yet highly inbreeding but not obligate breeding system.

X. italicum appears to have comparable levels of variation, in terms of polymorphic loci and per cent heterozygosity, to other inbreeders. Also there was an excess of heterozygotes compared to expected levels, at the 2 polymorphic loci as has been found in some other inbreeders [Marshall and Allard, 1970b; Allard and Kahler, 1971]. There is some indication of differentiation in allele frequencies in the 2 *X. italicum* populations though this could in part have been due to the different sample sizes. Geographic [Levin, 1975a; Brown *et al*, 1975] and micro-geographic differentation [Hamrick and Allard, 1972; Brown *et al*, 1974] have been shown to be characteristic of the genetic structure of natural populations of some plants.

However, the genetic structure of *Xanthium* populations is more in agreement with that put forward by Stebbins [1957] for facultatively self-fertilized species. He claimed that each species maintains itself as a number of genetically homozygous pure lines, with each line represented by hundreds of thousands of individuals. In this context a race of *X. strumarium* would "equate" to a pure line. An isolated population of *Clarkia franciscana* has been reported to be monomorphic

at nearly all loci [Gottlieb, 1973b], but for no plant species with wide geographic distributions and normal chromosomal systems has monomorphism at all loci been previously demonstrated. The prime reason for lumping of the races under the one species, *X. strumarium* for this study, is that they can hybridize at least with suitable manipulation of the photoperiod. *X. strumarium* then has a quite high proportion of polymorphic loci but a very low heterozygosity figure, yet these parameters are zero for the races, other than *X. italicum*, when considered individually, and also for *X. spinosum*.

Pertinent to this discussion is the question what factors limit the distribution of the races and species within Australia? Does the qualitative allozyme differences between races play a role in determining their distribution, or is this determined solely by places of original introduction, ecological factors and different colonizing strategies? Are the allozymes adaptive or neutral in these particular environments? From the initial survey of quantitative enzyme activity no obvious inferences could be made about the comparative activities of alleles at the segregating loci, and the distribution of the races fixed for these different alleles. Further studies are required to confirm this, but in very few cases have apparent selective differences between alleles been shown [Koehn, 1969].

Low genetic variation in other organisms has, seemingly in an *ad hoc* fashion, been explained by selection for homozygosity in an uniform environment [Nevo *et al*, 1974; Snyder, 1974]. However, all *Xanthium* species characteristically inhabit highly fluctuating environments. The genetic uniformity in populations of the colonizing land snail *Rumina decollata* [Selander and Kaufman, 1973a] in some respects closely parallels that of *X. chinense* and *X. spinosum*. Thus the lack of variation could be due to either selection for monogenic genotypes after

their introduction or the original introductions were restricted to unique genotypes or a third possibility is that there was genetic uniformity in the original native populations. Since most of the inbreeding colonizing species studied have been shown to have considerable amounts of genetic variation [Jain, 1969; Allard *et al*, 1968; Allard and Kahler, 1972a], it seems unlikely that there was selection for unique genotypes in all 3 races of *X. strumarium* as well as *X. spinosum* after their separate introductions. More likely, there was no allozyme variation in the original introductions, with the exception of *X. italicum*, but this indicates that, in particular, *X. spinosum* and *X. chinense* can colonize and occupy large geographic ranges without requiring allozyme variation. If this is correct it seems probable that the allozymes are selectively neutral, and genetic uniformity is maintained by random drift. It would be very interesting to determine the levels of allozyme variation in native populations of *Xanthium*.

Field observations suggest that there is morphological variation in natural populations of *Xanthium*. If there is quantitative variation it could either be environmentally determined (plasticity) or genetic in origin but a combination of both is more likely.

It is important then to determine (1) the nature and extent of quantitative variation in *Xanthium* populations, (2) what proportion of this variation is genetically determined, and (3) whether there is a correlation between the level of quantitative genetic variation and the amount of allozyme variation.

CHAPTER 4

QUANTITATIVE VARIATION

4.1. QUANTITATIVE VARIATION IN NATURAL POPULATIONS

The life cycles of flowering plants can be conveniently divided into the groups annual, biennial and perennial. A general scheme for the life cycle of a plant population is shown in Fig. 4.1. It is convenient to examine the important demographic events within the life cycle separately. A brief review of the relevant literature pertaining to the ecological and genetic aspects of these events in annuals, with special reference to inbreeding annuals, is made here. Of particular importance are the determinants of population variation in the adult phases of the life cycle and the variation in seed dormancy and germination processes (birth in demographic terms).

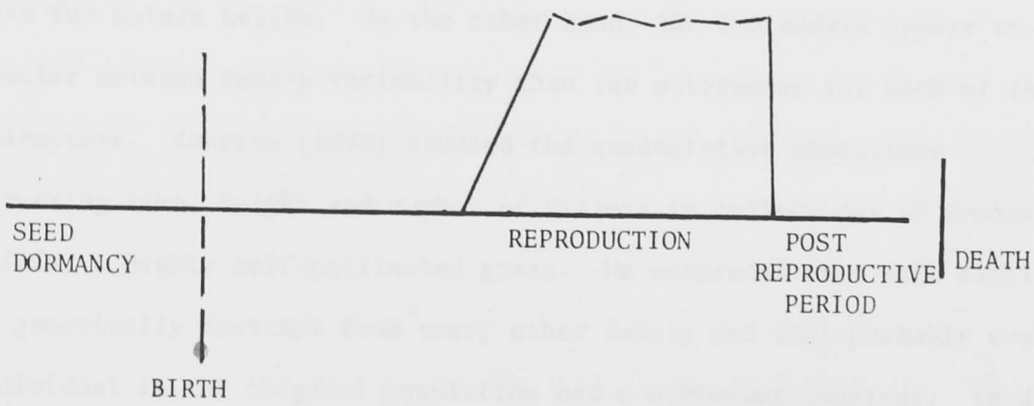


Fig. 4.1. Generalized life cycle of a plant population. Germination of seeds is equated with birth [after Harper and White, 1974].

It is a common observation that natural populations of annuals can contain considerable quantitative (or continuous) variation in morphological and physiological characters. Except for the early work of Knowles [1943], this variability had not been quantified until recently [Iman and Allard, 1965; Allard *et al*, 1968; Jain and Marshall, 1967; Lawrence, 1969; Hayward and Nsowah, 1969; Hillel *et al*, 1973]. The genetic component of this variability within a species can be divided into several parts: the variance between populations of a species, the variance between families within populations and the variance within families. The level of the environmental component of the variation is a measure of the plasticity that the species has for the particular character in question.

Is the mating system of a particular species a large determinant of the level of quantitative variability within it? Allard [1965] reviewed the population variability under various mating systems. He compared an inbreeder, wild oats, with annual ryegrass, a highly out-crossed annual grass. Within family variability is greater for the outbreeder than for the inbreeder for time of heading but about the same for mature height. On the other hand, the inbreeders appear to have greater between family variability than the outbreeder for both of these characters. Knowles [1943] studied the quantitative characters flowering time, height and number of tillers in collections of *Bromus mollis*, a highly self-pollinated grass. He concluded that each family is genetically distinct from every other family and that probably every individual in the original population had a different genotype. In a recent study of wild populations of the same species, Jain *et al*, [1970] found that measuresof within and between family components of variability indicated "a substantial genetic component of total variability in populations of this species." In contrast to Knowles' earlier work there was little evidence of geographic differentiation in *Bromus mollis*.

Jain and Marshall [1967] in a comparative study of variation in 2 *Avena* species obtained estimates of the coefficient of variation for panicle length and spikelet number, which showed variation among different sites within each of the species. Moreover, while both species were highly variable phenotypically, *A. barbata* had significantly larger phenotypic variation compared to *A. fatua*. In the *Festuca microstachys* complex, in which the mating system is enforced self-fertilization, Kanneberg and Allard [1967] showed that variability among families within a species was extensive at most sites. Further data for combinations of characters suggested that very large numbers of different genotypes coexist in each population.

Kanneberg and Allard [1967] also compared the amount of phenotypic and genetic diversity in ryegrass, wild oats and the highly inbreeding fescues, using as a measure the coefficient of variability. Results indicated that the fescues are as variable genetically as the other species. It would seem that there is no direct relationship between mating system and the extent of population variability. This is in contrast to earlier views on the comparative genetic structures of inbreeding and outbreeding populations. It was usually argued that inbreeding would increase genetic uniformity and hence allow the populations to achieve closer adaptation to the immediate environment, but at the expense of future adaptability.

Stebbins [1957] in a review of the populational variability of several less successful herbaceous self-pollinating species of California stated that "each successful biotype maintains itself as a constant, genetically homozygous pure line for a large number of generations and is represented by hundreds or thousands of individuals. It is normally isolated by self-fertilization from other biotypes of the same species with which it grows sympatrically." It is likely that the two views

represent the two limits of the spectrum and that both extremes could in fact be found within a single species [see Jain *et al*, 1970].

From the work done on the introduced annual grasses of California, it can be said there is little difference in total genetic variability between outbreeders and inbreeders, but inbreeders tend to be more variable between families and the outbreeders to be more variable within families. In natural populations of inbreeders there are quite high levels of phenotypic variation. Contrary to the above, however, Hillel *et al* [1973] who compared two closely related species of the wheat group, one being an inbreeder and the other, *Aegilops speltoides*, predominately outcrossing found that for the majority of the 36 quantitative characters examined, the differences between populations, the total variances of the populations and the mean within family variances were greater in the selfer than in the outbreeder. Most, but certainly not all, of the evidence for quantitative variation in annuals comes from work on monocots (grasses). What is the nature and extent of quantitative variability in natural populations of the species of *Xanthium* in Australia? Do the species have as much phenotypic variability as other inbreeding annuals? Is this quantitative variation purely environmentally determined and if not, what is the size of the genetic component of this variation? Is there a correlation between the amount of quantitative and qualitative genetic variation.

Ecology and Variation

The limits of phenotypic variation in natural populations of annual plants can be markedly determined by environmental and ecological parameters. The roles of competition and density in the juvenile and reproductive stages of plants have been well documented. Plants possess powers of reproduction, which permit, theoretically at least,

an exponential increase in the size of their populations, and yet, over a period of time, their natural populations vary between rather narrow limits.

There are two major ways in which plants respond to increasing density namely (1) a plastic response such that the reproductive capacity of an individual is reduced; (2) mortal response in which increasing density reduced the change of individual survival [Harper and Gajic, 1961]. The latter response was further subdivided by Marshall and Jain [1968] into (a) biological death, where the individual dies and (b) genetic death, in which the plant lives to the end of the season but produces no offspring. Harper and Gajic [1961] showed that populations of *A. grostemma githago* have the properties of self-regulating systems and that this is achieved largely through a plastic response, in which capsule number and the number of seeds per capsule were reduced by increasing density. In contrast, Harper and McNaughton [1962] showed *Papaver* species to exhibit predominately a mortal response to increasing density. *A. githago* is predominately self-fertilized, whereas *Papaver rhoeas* is wholly outbreeding. However, from a series of experiments with 2 *Avena* species it was found that "both species act primarily through a plastic response to density " [Marshall and Jain, 1968]. These data suggest that plastic responses to increasing density are maximized in inbreeders whereas mortality responses predominate in outbreeders.

Mortality responses (or self-thinning) in *Conyza canadensis* have been shown by Yoda *et al* [1963] to fit a so-called $\frac{3}{2}$ power law of self-thinning ($\omega^* = Cp^{* \frac{-3}{2}}$, where ω^* is the mean plant weight, p^* is the existing plant density and C is a coefficient with a characteristic value for a particular species). This gives a straight line of fixed gradient (-1.5) on the log ω^* - log p^* diagram. This relationship was

also found to hold for 2 other annuals, *Amaranthus retroflex* and *Chenopodium album*. White and Harper [1970] confirmed and extended this relationship to several other species. They also presented evidence that the smallest plants in a population are the first to thin leaving larger ones to grow more rapidly. Observation of *Xanthium* populations revealed an apparent range in densities. To quantify this, the density of several *Xanthium* populations have been determined, at the same time that morphological characters in these populations were measured. The aim was to determine how *Xanthium* species respond to increasing density.

In Australia edaphic and climatic components of the environment can vary enormously and can concomitantly determine the distribution of, and extent of variation in, field populations. Morley and Katznelson [1965] discussed how these have individually, and together, placed limits on the colonization of Australia by subterranean clover. Does climate have a selective effect on variation in *Xanthium* species colonizing Australia? Or does it limit the distribution and variation of the races and species without adaptation occurring in *Xanthium*.

For germination of both *X. strumarium* and *X. spinosum* late spring and summer rainfall is required. The time of germination and hence the length of the life cycle is usually determined by onset of these rains, especially in the drier inland areas. Early in the season temperatures are still quite low, so that seed dormancy is still enforced and the upper seeds do not germinate at this stage [Wapshere, 1974; Mann, 1965]. However, if the first rains are very late (after mid-November), or the suitable habitats are still flooded (a common occurrence in some seasons for *X. strumarium*), then both seeds could germinate within a couple of weeks of each other. Under such conditions competition for nutrients and density effects could come into play. Hence, even within a population of these annuals a range of age structures could occur

depending on moisture and temperature levels. Wapshere [1974] has shown that density, soil type and topography can interact to produce a wide range of variation in *X. chinense* plants within sites within a season. Variation within and between populations is clearly dependent on these climatic and edaphic factors.

X. strumarium is a short day plant being dependent on day length for flowering, and has a determinate growth form, in which "death follows seed set as if the act of seed production was itself lethal" [Harper and White, 1974]. Since the races of *X. strumarium* have different short-day requirements, and hence juvenile periods of different length, consequently they may have different potential ranges of phenotypic variation. *X. spinosum* on the other hand does not have a qualitative short day requirement. Harper and White [1974] questions "whether the length of the juvenile period has any significant effect on the potential rates of population growth in annual species." It could well critical for species of determinate growth form such as *Xanthium*, in which the length of the juvenile phase partly determines the level of reproductive output of the population.

Seed Dormancy and Germination

The literature on seed dormancy and germination in *X. strumarium* was reviewed in Chapter 2. No comparative work has been published indicating whether seed dormancy and germination requirements are the same or different for populations or races of *X. strumarium* and for *X. spinosum*. The distribution of the 2 species suggests that there could be differences. Mayer and Poljakoff-Mayber [1963] reviewed the literature relating to the ecology of germination. They enumerate the various external factors, such as water, soil type, temperature, gases and light that effect germination and the relation of these to the type of habitats encountered.

Do all the plants in a population of an annual have a characteristic requirement for germination? Certainly many agricultural crops do, though perhaps this has been selected for, but whether weeds and colonizing species have is uncertain. Harper [1965] showed that there was variation in germination requirements within and between populations of *Rumex* species. Thompson [1973] examined a number of species of the family Caryophyllaceae for their germination responses over a temperature range, and concluded that generally those modifications that do occur are likely to be restricted to small changes in part of the temperature range. He concluded that, at least for some weedy species, differences in germination responses between widely separated populations may be quite slight, and the response is an adaptation to the climate of the original geographic region. Real evidence for adaptation in germination behaviour to relatively new habitats (approximately 200 years) for recently colonizing species is very hard to find.

However, there are several examples of seed polymorphism in which the two or more forms have different germination requirements. Some examples are *Spergula arvensis* [New, 1961], *Rumex* [Cavers and Harper, 1966], *Chenopodium album* [Williams, 1962] and *Papaver dubium* [Arthur *et al*, 1973]. In some species this polymorphism is genetic (e.g. *Spergula*) but in others it is clearly somatic [reviewed by Harper *et al*, 1970].

Light has been shown to be a factor influencing germination in some plants, and it would be expected to be stimulatory to seeds germinating on the soil surface [Mayer and Poljakoff-Mayber, 1963]. It does not affect the germination of *Xanthium*. Wapshere [1974] showed that the germination of *X. strumarium* covered by sand in the field was three times greater than for seeds on the surface. Buried seeds are in a much wetter environment allowing water uptake to continue until

germination takes place. Sagar and Harper [1960] demonstrated a pre-chilling requirement for 2 *Plantago* species, but not for a third, and their field observations confirm this conclusion. Cooper and McWilliam [1966] found no requirement for chilling before germination in several Mediterranean populations of *Phalaris tuberosa*. The chilling requirement for germination has been compared to diapause in insects, and it could be argued that summer annuals are more likely to exhibit such phenomenon than winter annuals.

There are very few examples of the genetic control of dormancy or of the genetic regulation of germination. Morley [1958] from an analysis of F_2s and a diallel demonstrated that seed dormancy in *Trifolium subterraneum* was partly dependent on the genotype of the embryo. The evidence for genetic regulation of germination and the difficulties in showing such, have been reviewed by Whittington [1973]. As he pointed out, in order to determine the component of variability in time of germination either controlled crosses or deliberate selection experiments are required. Constant differences between races may suggest adaptation but cannot prove it.

Cohen [1967, 1968] in a series of theoretical papers introduced a formal framework to deal with the population biology of seeds. MacArthur [1972] extended Cohen's mathematical model of germination strategies to a model for delayed germination of desert annuals, a model which fits the *Xanthium* situation in many aspects. The population dynamics of the seed bank in the soil can be examined and the total seed population partitioned into several components. In one form this can be expressed as $S = G + ED + ID + D$, where S is the total seed population, G the germinated fraction, ED the number of seeds enforced in dormancy, ID the number of seeds induced in dormancy and D, decayed

TABLE 4.1.

DETAILS OF SITES OF *XANTHIUM* POPULATIONS SAMPLED FOR QUANTITATIVE CHARACTERS

Species	Population	N	Place	Date	Site Description
chinense	44	175	Windsor	29/ 9/74	Ridge of river flat of Hawkesbury, only annuals present
	50	100	Raymond Terrace	30/ 9/74	Hunter River flats, fenced paddock - summer grasses
	53	100	Binnaway	4/12/74	Edge river bank of Castlereach, overgrown with grass species
	55	92	Darlington Point	14/ 4/74	Banks overflow lagoon of Murrumbidgee, partly shaded
	58	102	Hay	16/ 4/74	Murrumbidgee River bank - heavily shaded by Eucalypts, other plants rare
italicum	46	60	Muswellbrook	30/ 9/74	Hunter River bank, several grass species present
	52	100	Sandy Hollow	3/12/74	Disturbed area - bare, gravelly sand, bank Goulburn River
pennsylvanicum	59	100	Mildura	15/ 4/74	Murray River flats - sandy soil, other species rare except Eucalypts
spinosum	56	72	Hay	15/ 4/74	Disused horse paddock, grey clay coil, *spinosum* dominant
	57	84	Hay	15/ 4/74	Dry grey soil, several annuals present, 200 metres from Murrumbidgee.

seeds. Sarukhan [1974] used this breakdown of the total seed population in his extensive experiments on seed populations of *Ranunculus* species in the field.

In this Chapter experiments are described, aimed at determining the variation in germination requirements and in the amount of germination under various temperature regimes, for populations of *X. spinosum* and the races of *X. strumarium*. An attempt was made to quantify the seed dormancy in *Xanthium* at a population level.

4.2. MATERIALS AND METHODS

4.2.1. Field Measurements

Population sites at which densities and quantitative characters were measured are described in Table 4.1. In Figures 4.2. and 4.3. several of these sites are illustrated. From Table 4.1., it can be seen that there are two separate times at which this data was collected. Five populations of the 1973/74 growing season were measured just prior to the start of the 1974/75 season. This has the disadvantage of giving little information about mortality responses (and to a lesser extent plastic responses) to density in the actual growing season, and of underestimating the number of fruit/plant character. In compensation, it does give some estimate of the fraction of the seed load that does not germinate in the first season simply because seeds have not reached the ground. The other 5 populations were measured in late summer of the 1974/75 growing season. Except for *X. cavanillesii*, at least 1 population of each race and species were measured. *X. cavanillesii* plants in population 44 were sampled for quantitative characters, but there were too few to be included in the analyses. It is estimated that there would be less than a 1000 plants of this race growing in any one season in Australia.

Fig. 4.2. Two population sites sampled for quantitative variation

(a) Hay (56) - *X. spinosum*

(b) Mildura (59) - *X. pennsylvanicum*

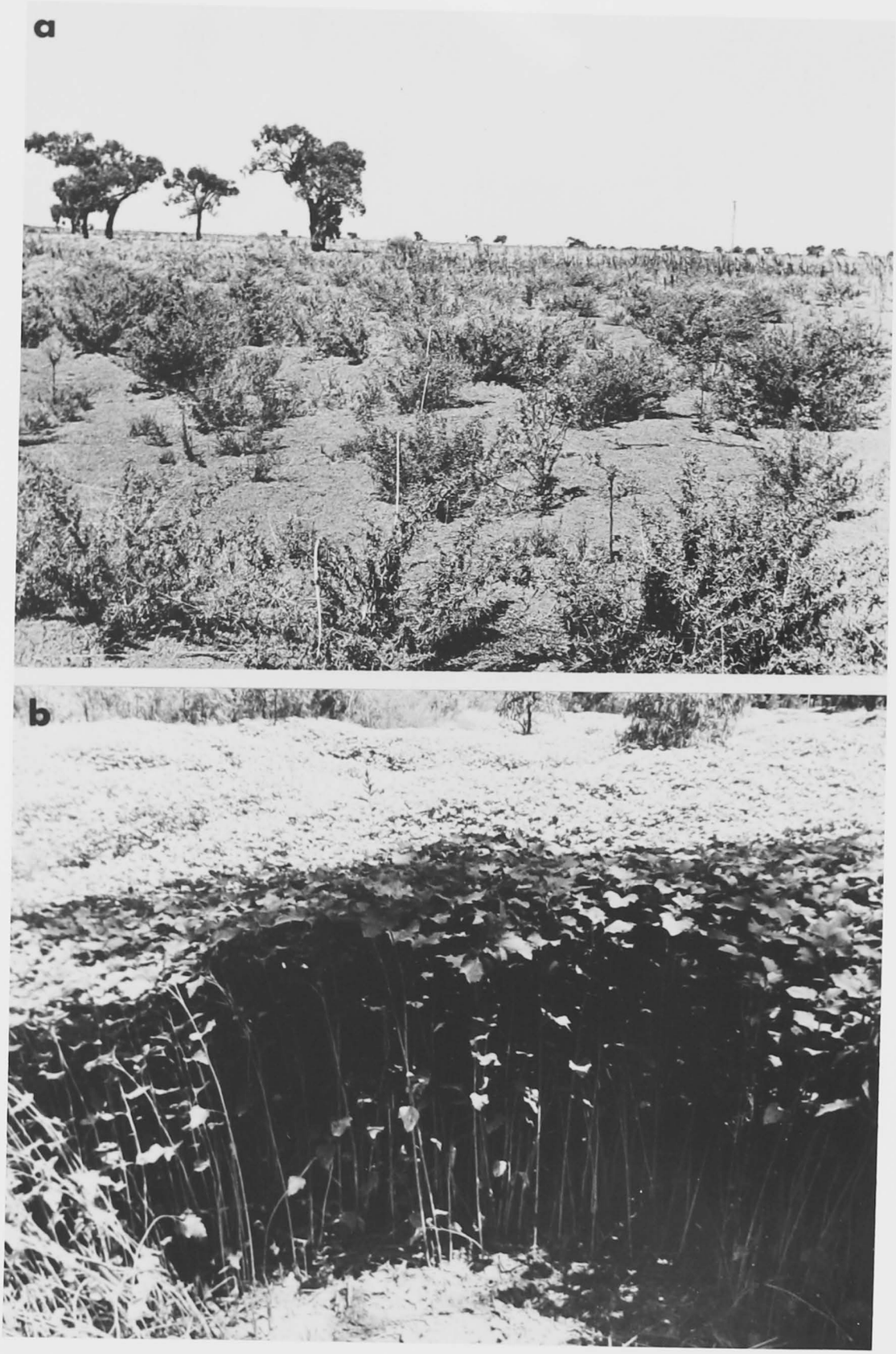

a
b

Fig. 4.3. Two population sites sampled for quantitative variation

(a) Hay (58) - *X. chinense*

(b) Muswellbrook (46) - *X. italicum*


a


b

For 8 of the populations, density was determined by the point-centre quarter method [Greig-Smith, 1964]. Distances were obtained by laying down parallel transects 30 metres long and 4 or 5 metres apart and then sampling at randomly determined distances along these transect lines. The other 2 population sites (44 and 53) consisted of narrow stands of *Xanthium* along ridges of river banks and were measured by the linear transect method in which the positions of all plants on either side of a transect through the centre of the population (parallel to the river bank) are recorded. There is great patchiness in many stands of *Xanthium*, often correlated with variations in the terrain. This is less so for *X. spinosum* since it occurs more in disused cultivations than in association with river systems. To this extent, there was selection of stands of *Xanthium* on fairly regular ground.

Quantitative characters were measured on the same plants as those used in obtaining the density measurements, so that in fact a fine grid of the populations for one or all of the characters could be mapped. If anything the densities obtained by the point-centre quarter method are an underestimate [Harper, 1960]. Because of the criteria employed in choosing sites, it is considered that the estimates are not seriously in error. All characters were measured in the field, except for fruit length and fruit weight. For these, a sample of fruit was obtained from each plant and 5 fruit from each plant were measured for both characters. This gave a measure of within and between family variability for these characters.

As part of preliminary work of experiments in Chapter 5, data on seed weights for several half-sib families in 7 populations were collected. These data are only for the races of *X. strumarium*, since seeds cannot be extracted intact from fruit of *X. spinosum*. Five of the populations are different from the ones sampled for the other

quantitative characters, enabling further comparison to be made on levels of phenotypic variability in *Xanthium* populations.

The percentage seed set measures how many fruits have 2 developed seeds. This measure was determined by taking a random sample of at least 100 fruit from a population and cutting them open. Absence of seed was marked by an empty testa.

4.2.2. Germination

Seed stocks used in these experiments were stored dry at room temperature for at least 6 months. The fruit were from random general collections made at the same time as populations were sampled for qualitative analysis.

Preliminary experiments indicated that perlite gave the optimum percentage germination (compared to peat, sand and soil) with fruit placed a ¼-½ an inch below the surface. A seed was classed as having germinated when the root tip had emerged from the fruit and the base of the cotyledons had become visible. For all experiments, the number of seed germinated was scored every 2 days, and scoring was complete when no seeds had germinated for at least a week (usually 2 weeks). In all cases, the basic parameters derived were the percentage germination, the rate of germination and the level of dormancy of the upper seed. Seeds were kept in the dark except when scoring was in progress. Seeds, classed as germinated, were removed from replicates and the fruits were replaced in such a way that fruits with 1 and 2 seeds could be distinguished. Normally this was done by painting the tip of the beaks of the fruit with 1 seed left.

Incubators were used to get a range of fixed temperatures and the temperature stated for a particular experiment was the air temperature within the incubator. It was not possible to test the more realistic situation of alternating temperatures under day/night regimes.

To test for a prechilling (stratification) requirement fruit were placed in moistened peat in plastic bags at 4°C. Two experiments were carried out to test the effect of chilling on germination. A small-scale experiment, with 2 replicates of 20 fruit each, was designed to see if the length of the chilling period (0, 3, 7, 14, 21 days) had any effect on the percentage germination. In another series of experiments, 3 replicates of 50 fruit from 5 populations were stratified for 2 weeks at 4°C before transferring to the known optimum germination temperature for the species involved. This enabled direct comparisons to be made with results from the main experiment.

The aims of the main experiment were twofold (1) to measure the percentage germination, the rate of germination and the amount of seed dormancy in the races and species of *Xanthium* over a temperature range and (2) to categorize the seed populations into their various components. The viability of seeds was tested using 2, 3, 5-triphenyltetrazolium chloride [Moore, 1973].

In the main experiment each population was represented by 3 Reps of 50 fruit at each of the 4 temperatures. In the first phase, the initial fraction (G_1) of the seed population, that germinates in the first 3 weeks, simulates the flush of germination following early spring-summer rains. After this phase, fruits were left to dry in the incubators, then seeds were slightly scarified and placed in moist conditions for a further 3 weeks to simulate the germination in the rest of the season (G_2). The sum of these phases is the fraction that germinated during the course

of the experiment (G_n) and was equivalent to the overall germination in the field in a favourable season.

The components of the non-germinating fractions (R) for the present analysis were (1) ED = dormant enforced seeds, which germinated when removed from fruit and placed at 35°C on moist filter paper, (2) ID = dormant induced seeds that did not germinate on filter paper but were viable by the tetrazolium test, and (3) the D (dead) fraction consisting of the non-viable, decaying and empty seeds. These components of R were only estimated for treatments at 30°C and 35°C.

Finally, the variation in germination behaviour within half-sib families was examined by taking 5 half-sib families at random from each of 6 populations (3 of *X. chinense* and 3 of *X. spinosum*). There were 2 replicates of each half-sib family. The size of these replicates were 15 fruit and 20 fruit for *X. chinense* and *X. spinosum* respectively, size being limited by the availability of fruit.

4.3. RESULTS

4.3.1. Field Populations

The phenotypic variation in populations of *Xanthium* is shown by the data in Tables 4.2. and 4.3. In Table 4.2., the means and coefficients of variation for the characters plant height, number of branches and number of fruit of 10 populations are listed. Details of the populations are given in Table 4.1. and Figs. 4.2., 4.3. and 4.4. There are marked differences in means for plant height and number of fruit between the populations of *X. chinense*.

The plants can have markedly different morphological forms. For example, populations 55 and 50 have similar means for plant height yet population 50 consists entirely of single-stemmed plants with no branches, while in population 55 the plants commonly have several branches. Also,

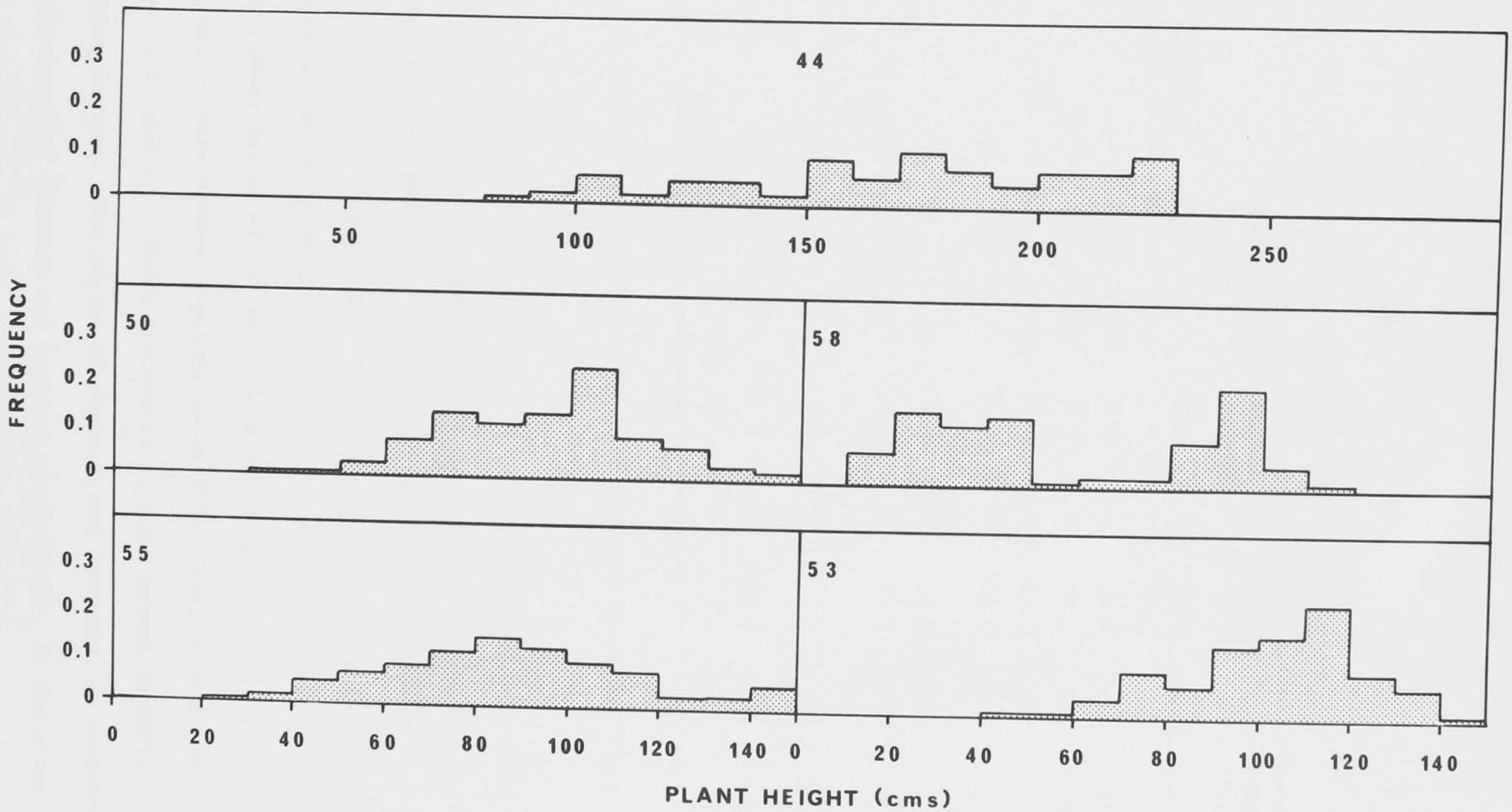


Fig. 4.5 Distributions of Plant Heights in X. chinense populations

TABLE 4.2.

PHENOTYPIC VARIATION IN NATURAL POPULATIONS OF *XANTHIUM*

Populations		Plant height (cm) Mean	C.V.	Number of branches Mean	C.V.	Number of fruit Mean	C.V.	Density (Plants x m^{-2})
chinense	53	102.1	0.20	8.4	0.70	51.6	1.31	1.01
	58	58.8	0.51	0.0	0.00	6.7	1.06	102.00
	55	91.5	0.33	0.0	0.00	27.3	1.08	33.33
	44	171.2	0.23	9.8	0.90	18.8	1.82	0.90
	50	93.8	0.22	6.2	0.60	52.8	0.55	0.19
italicum	46	38.1	0.61	1.6	1.71	19.8	1.36	0.67
	52	37.2	0.61	2.8	2.07	22.2	1.47	0.90
pennsylvanicum	59	63.7	0.33	0.0	0.00	10.9	0.77	123.46
spinosum	56	58.5	0.38	28.1	1.22	523.0	1.36	0.92
	57	31.6	0.50	0.0	0.00	11.1	0.72	15.90

plants in 55 have only approximately half the mean number of fruit compared to those in 50.

The estimates of the coefficients of variation indicate quite high levels of phenotypic variation within and between populations of *X. chinense*. The amount of variability (size of C.V.) appears to be characteristic of the particular trait concerned. Thus there are high levels of variation in fruit number within populations of *X. chinense*, but much lower levels of variation in plant height. The distributions of characters vary between populations as illustrated by plant height in Figs. 4.5. and 4.6. These distributions fit a normal distribution to varying degrees. The degree of skewness and kurtosis, as measured by coefficients of skewness and kurtosis respectively, in the plant height distributions are shown in Table 4.4. Seven of the populations have some positive skewness, but only 2 of these are significant (the 2

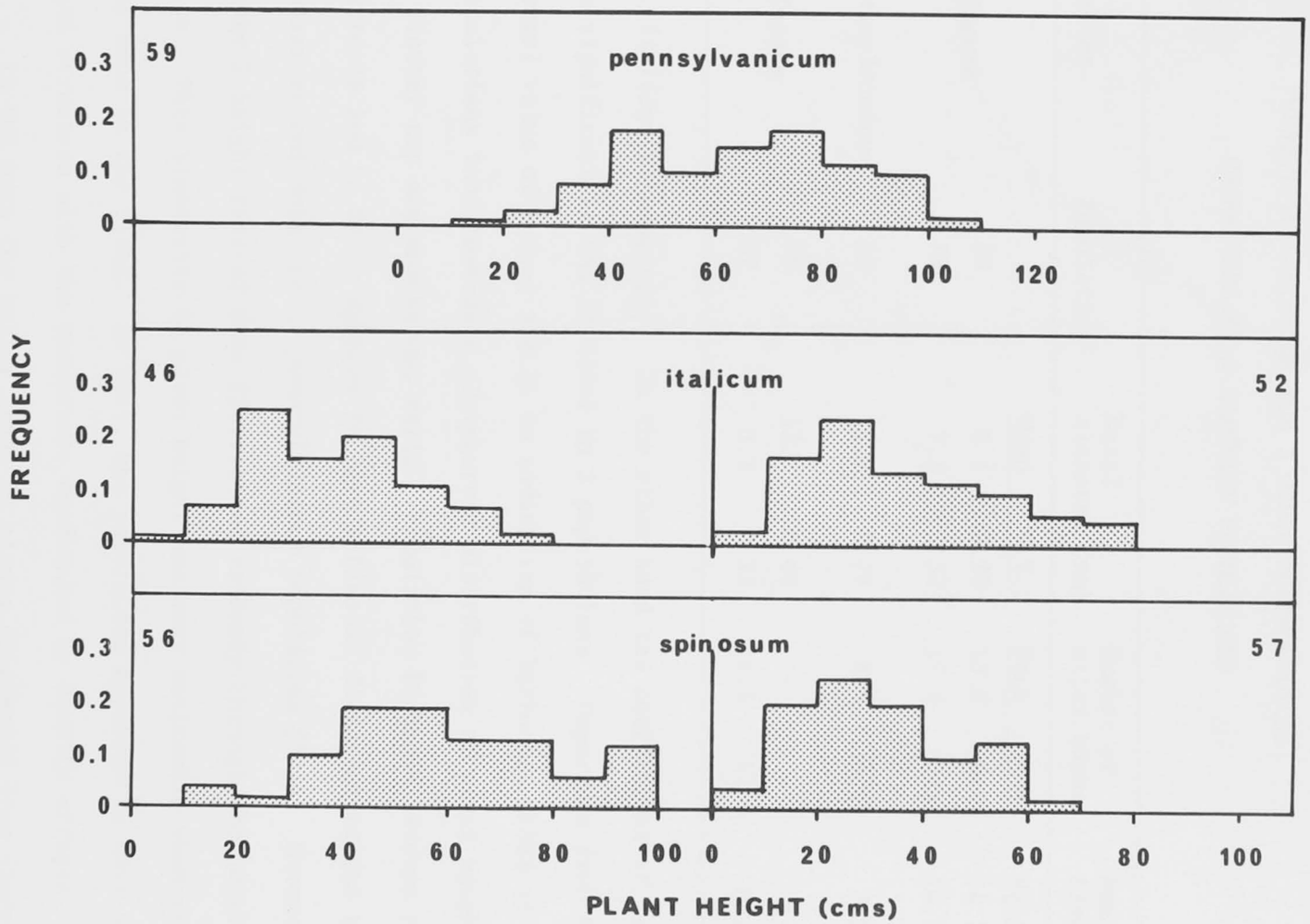


Fig.4.6 Distributions of Plant Heights in Xanthium populations

TABLE 4.3.

PHENOTYPIC VARIATION FOR 3 OTHER MORPHOLOGICAL CHARACTERS IN 5 *XANTHIUM* POPULATIONS

Species	Population	Basal diameter (mm)		Number of major leaves		Number of male flower clusters	
		Mean	C.V.	Mean	C.V.	Mean	C.V.
chinense	58	5.1	0.29	13.0	0.22	4.7	0.79
	55	7.3	0.32	17.6	0.41	14.1	1.07
pennsylvanicum	59	5.9	0.28	8.6	0.61	7.9	0.57
spinosum	56	12.2	0.44	-	-	-	-
	57	3.3	0.32	16.4	0.29	5.1	0.75

X. italicum populations). On the other hand the coefficient of kurtosis is significant at the 1% level in 3 populations. Departure from the normal value of $b_2(\gamma_2) = 3$ is an indication of kurtosis. Most of the populations tend towards a platykurtic distribution of plant height with a flatter top and more abrupt tails. Population 58 shows extreme platykurtosis and in fact, appears to have a bimodal distribution (as to a lesser extent does the *X. pennsylvanicum* population [59]). However, these 2 height classes were distributed randomly through the population site. This bimodality in plant height was very noticeable when scoring was done in the field. In fact, the population appeared to have a two-aged structure with the structures confounded by density effects.

In the main the taller group of plants originated from lower seeds, and the small plants from upper seeds of the fruit. This was demonstrated by analysing the fruit population contained in a square metre of soil in the middle of population 59, for seed content. The fruit are normally attached to, or entangled within, the root system

TABLE 4.4.

THE COEFFICIENT OF SKEWNESS (γ_1) AND COEFFICIENT OF KURTOSIS (γ_2) VALUES FOR THE DISTRIBUTIONS OF PLANT HEIGHTS IN THE 10 POPULATIONS

Populations	N	γ_1 $^{\phi}$	γ_2 $^{+}$
53	100	-0.470	2.80
50	100	-0.262	2.97
44	169	-0.217	2.03**
55	92	0.250	2.71
58	102	0.133	1.40**
46	60	1.67**	2.35
52	91	1.39**	2.42
59	99	-0.051	2.12**
56	68	0.170	2.47
57	84	0.505	2.49

** Significant at 1% probability level

$^{\phi}$ Table 34.B, Pearson and Hartley (1965)

$^{+}$ Table 34.C, Pearson and Hartley (1965)

just below the soil surface. The results are shown below.

Number of fruit/m^2 with			Seeds remaining	
2 seeds	1 seed	none	% original	% viability
0	108	38	37	90

Nearly all the small plants had no seeds in the corresponding fruit, while the tall plants had fruit with 1 seed. None of the fruit had 2 seeds intact, but the vast portion had 1 seed left, while 26% of the fruit were empty. This could mean that, in the early stages of the life cycle, the seedling population consisted of about 21% plants from upper seeds and 79% from lower seeds (this is without correcting for amount of seed set and other factors - see Table 4.14).

For population 58 the high C.V. value for plant height compared to the other *X. chinense* populations is explained by the 2 distinct groups within the population site. This bimodality within population 58 is not clear from the distribution of the other characters in this population. Not surprisingly, if population 58 is divided into 2 groups on the basis of plant height then the distribution of the other characters between these 2 groups differ quite markedly.

From Tables 4.2. and 4.3. it can be seen that there is considerable variation within populations of the other species. The 2 *X. spinosum* populations are very different in their means for the 4 characters that can be compared. The estimates of the coefficients of variation are larger for 3 out of the 4 characters in population 56 compared to 57. The large differences in the size of means shown for *X. spinosum* from these 2 populations give some indication of the enormous differences possible between populations within the same season. *X. strumarium* is capable of such large variation in growth also, but large stands of big

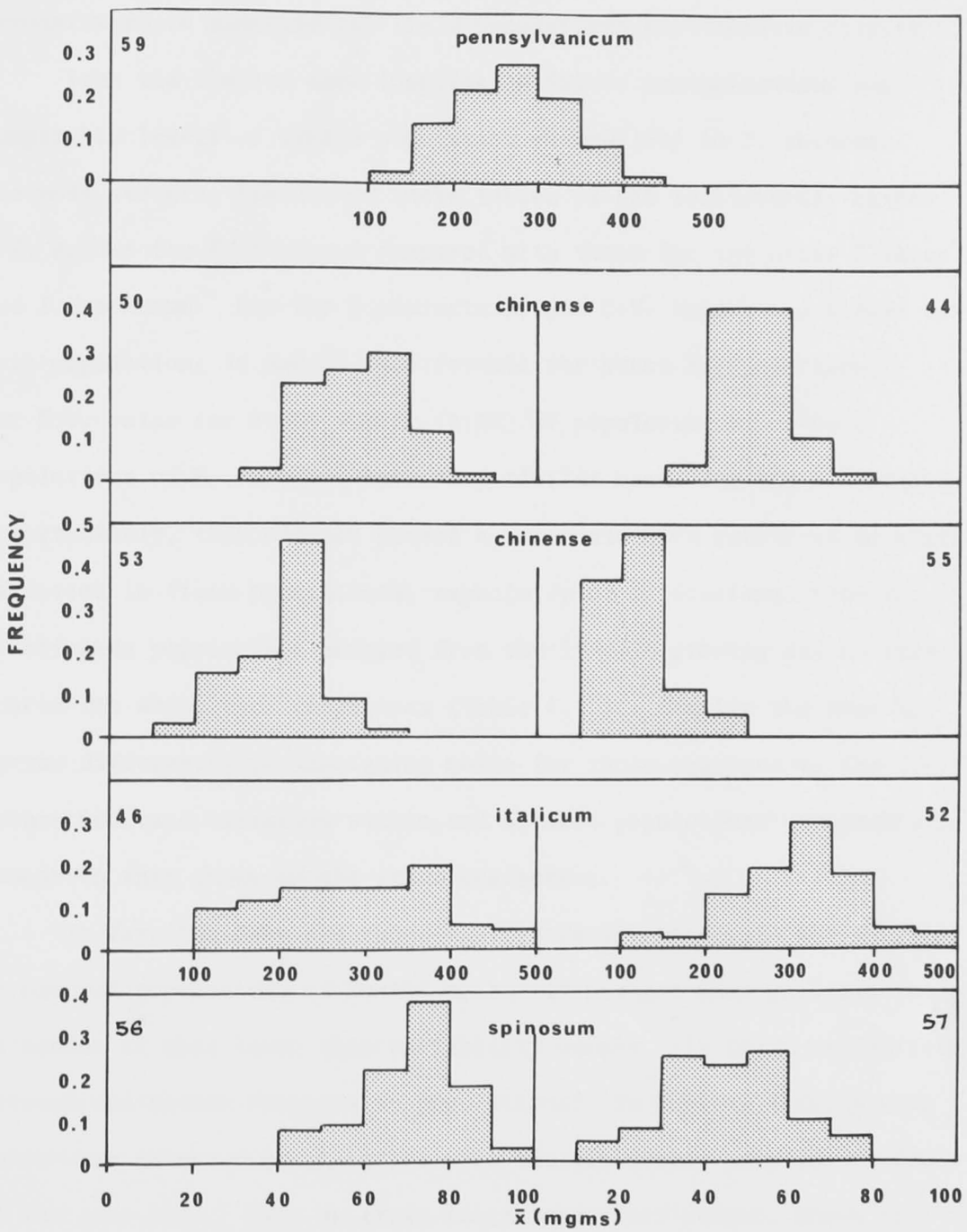


Fig. 4.7 Distribution of Family means ($\bar{x}_f$) of Fruit Weight at the population sites.

plant are rare. The very large size of individual plants possible is demonstrated in experimental field populations described in Chapter 5.

From the limited data (population 59) *X. pennsylvanicum* has comparable levels of within population variability to *X. chinense*. The most striking feature of these tables is the consistently higher C.V. values for *X. italicum* compared with those for the other 2 races and *X. spinosum*. For the 3 characters, the C.V. values are higher for both populations 46 and 52 than for all the other 8 populations, for C.V. value for fruit number (1.82) of population 44. The 2 populations of *X. italicum* have very similar means for the 3 characters. Unfortunately, time did not permit a more extensive survey of quantitative variation in field populations, especially of *X. italicum*. The 2 *X. chinense* populations sampled from the 1974/75 growing season were scored for additional characters (Table 4.3.). Despite the somewhat narrow differences in population means for these characters, the C.V. values indicate variation within and between populations comparable in amount to that shown by the other characters.

So far, the data has shown that there is phenotypic variability in natural populations of *Xanthium*, but it has not been possible to determine at what level this variability occurs. Is there variability between and within families in populations? To explore this, plants which were measured quantitatively in the field were sampled for fruit at the same time. Data on fruit length and fruit weight, shown in Tables 4.5. and 4.6, demonstrate phenotypic variation within populations and show that this variation is both within families and between families. Although both species are self-compatible, the data can only be said to be from half-sibs with certainty, so no conclusions can be made about the genetic component of the variation. The distributions within populations of family means for fruit weight are shown in Fig. 4.7.

From the ranges of family means for both characters, and the distributions of fruit weight means, it can be seen that there is a great deal of phenotypic variability among families within all populations. The complete difference in appearance of the fruits of the 2 species is reflected in the much smaller fruit length and weight values for the 2 *X. spinosum* populations.

The S_{ω}^2 values of fruit length were in most cases lower than the corresponding S_b^2 values. On the other hand the values of S_{ω}^2 for fruit weight were greater than the corresponding S_b^2 values for 4 of the 9 sites. For both characters, with respect to S_b^2 and S_{ω}^2 , the 2 *X. italicum* populations (46 and 52) exhibited greater variation than almost all the other populations. Moreover, the C.V. estimates for fruit length are larger for the *X. italicum* populations than for the other populations, but the $C.V._b$ and $C.V._{\omega}$ estimates for fruit weight, although larger than most corresponding C.V. estimates of the other populations do not present a clear cut picture. For both fruit length and fruit weight in both populations the $C.V._b$ estimates are larger than the $C.V._{\omega}$ estimates, although the differences are much larger for 46 than 52. Overall the C.V. values suggest that there is greater variation between families than within families.

To examine whether the C.V. values are significantly different from the different populations for all the various characters, van Valen's modified F test was employed. The problems involved in comparing C.V. values have been discussed by van Valen [1965], Lewontin, [1966) and Jain and Marshall [1967]. The difficulty is that there is no statistical test for comparing C.V.s from different samples. Here the ratio of the squared C.V.s has been calculated and this corrected F value looked up

TABLE 4.5.

ESTIMATES OF BETWEEN -AND WITHIN- FAMILY VARIABILITY IN FRUIT LENGTH

Population	Range of family means	Grand mean	Fruit Length (mm) S_b^2	d.f.	S_w^2	d.f.	C.V.$_b$	C.V.$_w$
53	15.2 - 22.0	18.7	1.55	77	1.62	312	.067	.068
55	14.0 - 20.0	17.7	1.66	59	0.75	240	.073	.049
44	18.0 - 22.0	19.8	0.63	94	0.44	380	.040	.033
50	19.0 - 23.0	20.7	0.94	85	0.72	340	.047	.041
46	14.3 - 25.0	20.7	5.55	58	1.58	236	.114	.061
52	17.0 - 25.2	20.2	2.63	73	2.07	296	.080	.071
59	19.0 - 23.4	21.7	0.85	87	0.76	352	.042	.040
56	9.2 - 12.0	10.4	0.35	65	0.37	264	.057	.058
57	8.5 - 12.0	10.1	0.59	72	0.27	308	.076	.052

in F tables for the appropriate degrees of freedom. Thus Table 4.7. shows the F values for some of the possible pairwise comparisons of populations considering 1 character at a time.

There is a marked separation of the data in Table 4.7. into 2 groups. On the one hand the F values for comparisons between the *X. italicum* populations are not significant at the 5% level for 2 out of the 4 characters and the other 2 values are significant at the 1% level. However, for comparisons of other populations within races, nearly all the F values are significant at the 5% level and most are at the 1% level also. Differences in levels of phenotypic variability between populations of *X. chinense* are significant as shown by the pairwise comparisons. Similarly the 2 *X. spinosum* populations are

TABLE 4.6.

ESTIMATES OF BETWEEN -AND WITHIN- FAMILY VARIABILITY IN FRUIT WEIGHT

		Fruit weight (mg)						
Population	Range of family means	Grand mean	S_b^2	d.f.	S_w^2	d.f.	C.V.$_b$	C.V.$_w$
53	101.4 - 315.8	205.5	2519	77	4002	312	.245	.308
55	55.0 - 226.8	118.8	1373	59	864	240	.312	.247
44	180.0 - 343.4	227.2	1130	94	1199	360	.132	.136
50	182.5 - 431.2	290.3	2669	86	2886	340	.178	.185
46	107.7 - 462.2	284.5	10565	58	4325	236	.361	.231
52	107.0 - 477.8	306.5	6201	73	5813	296	.257	.249
59	116.0 - 452.0	269.3	4590	87	4410	352	.252	.247
56	42.4 - 100.6	71.5	142	65	220	264	.167	.207
57	19.5 - 73.0	45.9	198	72	107	308	.307	.225

significantly different. The several probability values obtained for each population comparison could be combined using the P_λ test to give an overall estimate, based on all the characters, of whether the levels of variability differ significantly for the 2 populations concerned. Statistically this is not rigorously correct, since the P values should be from independent tests of significance. The χ^2 values calculated by combining probabilities ($-2[2.303]\Sigma \log P_i$) are significant for all comparisons ($P < .001$).

TABLE 4.7.

F^{ϕ} VALUES FROM PAIRWISE COMPARISONS OF POPULATIONS WITHIN SPECIES FOR 4 CHARACTERS

Population		Plant height	Number of fruit	Fruit length	Fruit weight
italicum	46 52	1.00	1.17	2.03**	1.97**
spinosum	56 57	1.73*	3.57***	2.00**	4.94***
chinense	55 58	2.39***	1.04	1.96**	1.65*
chinense	53 58	6.50***	1.53*	1.66*	1.02
chinense	58 44	4.92***	2.95***	1.69*	3.39***
chinense	55 44	2.06***	2.84***	3.33***	5.59***
chinense	53 44	1.32*	1.93***	3.06***	3.45***
chinense	53 55	2.72***	1.47*	1.19	1.62*

$^{\phi}$ Van Valen's corrected F-test based on the ratio of the $C.V._b^2$ (Van Valen, 1965).

*, **, *** Significant at the 5%, 1% and .1% probability level respectively

In summary, there is significant variation between populations within races with perhaps less variation between the *X. italicum* populations than between populations of the other races. The next point to consider is whether the amounts of variation in the races and the 2 species are different. The Mann-Whitney U test was used to test if (1) *X. italicum* is phenotypically more variable than *X. chinense* for the sites studied; (2) *X. italicum* is significantly more variable than all other types combined and (3) *X. spinosum* phenotypically more variable than *X. strumarium* (Table 4.8.).

Despite the low degrees of freedom, it can be seen that *X. italicum* is significantly more variable than *X. chinense* for 4 out of the 5 characters and that it is also significantly more variable phenotypically than all *X. chinense*, *X. pennsylvanicum* and *X. spinosum* combined for 3 out of the 5 characters. On the other hand, *X. spinosum* is not significantly more variable than the 3 races of *X. strumarium*. The χ^2 values calculated for the P_λ test (Table 4.8.) show that, if all characters are considered together, *X. italicum* is phenotypically more variable than *X. chinense*, *X. pennsylvanicum* and *X. spinosum* ($P < .001$).

Alternatively, the suspected greater variability in *X. italicum* populations can be examined by assigining relative ranks to each population for the C.V. estimates (Table 4.9.). The mean rank values show clearly that the *X. italicum* is more variable than the 2 races of *X. strumarium* and *X. spinosum*.

Data on variation in seed weight for several different populations to those studied above show the same trends with C.V. estimates for *X. italicum* higher than all others (Table 4.10). Although seed weight is significantly correlated with fruit weight ($r = .760$), these results are an independent confirmation of previous conclusions in that

TABLE 4.8.

THE PROBABILITY VALUES FOR MANN-WHITNEY U TESTS FOR COMPARISONS OF C.V. VALUES OF THE 5 CHARACTERS, AND THE Chi-SQUARES FOR THE P_λ TEST

Comparison	Plant height	Number of branches	Number of fruit	Fruit length	Fruit weight	χ^2 values
italicum *chinense*	.05	.05	> .10	.05	.05	73.00***
italicum other species	.025	.025	> .10	.025	.10	42.38***
spinosum *chinense*	> .10	> .10	> .10	> .10	> .10	< 23.03^{NS}
spinosum *strumarium*	> .10	> .10	> .10	> .10	> .10	< 23.03^{NS}

*** Significant at .1% probability level
NS Not significant at 1% level

seed were not from the fruit or plants previously analysed in population 46, and also in *X. italicum* that is being compared to 3 new populations of *X. chinense* and 2 of *X. pennsylvanicum*. The differences in means of upper and lower seed weights within populations are in the range of 10-20 mg, but there is an overlap in the distributions of upper and lower seed weights within a population. Although there is variation between sites for both *X. chinense* and *X. pennsylvanicum*, the $C.V._b$ estimates are within a common but quite narrow range. The C.V. estimates for *X. cavanillesii* are low compared to the C.V. values for the other races, and suggests, perhaps, that variability in this race could be lower than in the other 3. Another point to emerge from this Table is that the races have characteristic seed weight means with

TABLE 4.9.

RELATIVE RANK (HIGHEST TO LOWEST) OF EACH POPULATION FOR THE C.V. ESTIMATES

Population	Height	Number of branches	Number of fruit	Fruit length		Fruit weight		Mean rank
				$C.V._b$	$C.V._w$	$C.V._b$	$C.V._w$	
53	10	5	5	5	2	6	1	4.86
50	9	6	10	8	7	8	8	6.86
44	8	4	1	10	9	10	9	6.00
55	6.5	8.5	6	4	6	2	3.5	5.21
58	3	8.5	7	7	-	7	-	6.42
46	1.5	2	3.5	1	3	1	5	2.43
52	1.5	1	2	2	1	4	2	1.93
59	6.5	8.5	8	9	8	5	3.5	6.93
56	5	3	3.5	6	4	9	7	5.36
57	4	8.5	9	3	5	3	6	5.5

X. cavanillesii having the highest, followed by *X. pennsylvanicum*, with *X. chinense* and *X. italicum* being lower than the other 2 but about equal to one another.

In Table 4.11 F ratios and corresponding significance levels for pairwise comparisons of populations for seed weights are given. The *X. chinense* populations are not significantly different from one another at the 5% level, nor are the *X. pennsylvanicum* populations. *X. italicum* is significantly different from the least variable race, *X. cavanillesii* at the 5% level. Seed size, within a species, shows great stability in contrast to the enormous variation which occurs between species. In contrast to the high plasticity of other organs of the plant the

TABLE 4.10.

VARIATION IN NATURAL POPULATIONS FOR WEIGHT OF LOWER (A) AND UPPER (B) SEEDS

Population		A (Lower)		B (Upper)		Total*	
		Grand mean (mg)	C.V.	Grand mean (mg)	C.V.	Grand mean (mg)	C.V.
chinense	22	52.4	.146	34.5	.164	43.4	.153
	11	41.9	.183	29.5	.156	36.1	.158
	42	36.0	.211	22.4	.199	29.1	.203
italicum	46	46.2	.256	37.1	.271	41.6	.263
pennsylvanicum	25	61.0	.170	45.2	.185	53.0	.177
	26	64.3	.194	44.5	.162	54.3	.178
cavanillesii	44	103.3	.142	81.4	.141	92.3	.140

* The 2 seeds of each fruit combined

the stability in seed size seems to be a homeostatic device ensuring survival to the next generation [Harper *et al*, 1970]. However, the data clearly show that there is a seed polymorphism in *Xanthium*, where the 2 seeds of each fruit are of disparate size. It appears likely that this fixed variation is determined largely by the maternal genotype which specifies the structure of the fruit. Under certain circumstances density can have an effect on seed weight [Hodgson and Blackman, 1957] but in the vast majority of cases seed weight is maintained over a very wide range of densities.

From Table 4.2. it can be seen that there is a range of population densities from 1.9×10^{-1} to 1.23×10^{2} plants per square metre. Density regulates plant populations by either a plastic or a mortal response, or more commonly by a combination of both. With all other environmental

TABLE 4.11.

F^{ϕ} VALUES FROM PAIRWISE COMPARISONS OF POPULATIONS FOR BETWEEN-FAMILY VARIABILITY IN SEED WEIGHTS

Population		A Seeds	B Seeds	Total Seeds
chinense	22 11	1.57	1.11	1.07
chinense	22 42	2.09	1.47	1.76
chinense	11 42	1.33	1.65	1.65
pennsylvanicum	25 26	1.30	1.30	1.01
italicum *cavanillesii*	46 44	3.24*	3.69*	3.53*

ϕ van Valen's corrected F-test based on the ratio of the $C.V.^2$ (van Valen, 1965).

* significant at the 5% probability level

factors fixed, increasing density would be expected to lead to more uniform populations and concomitantly C.V. values would decrease. The results of Wapshere [1974] indicated that this density was about 1-2 plants per square metre for *X. chinense*. Below this figure edaphic, nutrient and water conditions will be the dominant determinants of levels of environmentally induced variation.

Controlled field experiments in which a range of densities are used, are required to determine whether C.V. values do decrease with increasing density or not. If just the *X. strumarium* populations are

TABLE 4.12.

MEAN RANKS OF 4 POPULATIONS FOR ESTIMATES OF C.V.

AND THE CORRESPONDING POPULATION DENSITIES

Population	Density	Mean Rank
53	1.01	1.50
55	33.0	1.72
58	102	2.86
59	123	2.84

considered, and only those in which density is high enough to have a regulatory role (59, 58, 55 and perhaps 53), a rank of these can be made as in Table 4.12. The mean rank values suggest a trend of decreasing C.V. values with increasing density. This would suggest that density does have an effect on the level of phenotypic variability within populations.

Wapshere [1974] demonstrated that in *X. chinense* height was fairly constant over a range of densities under fixed growth conditions but that different heights were obtained under different growth conditions. Similarly, under largely density independent conditions populations 53 and 44 have very different plant height means but similar densities while populations 55 and 50 have nearly identical plant height means, but there is a 165 fold difference in population densities (Table 4.2.).

Other less obvious environmental factors can contribute to the phenotypic variability between populations. Populations could have life cycles of different lengths as a result of different times of germination within the season. The Murrumbidgee and Murray River systems were subject to heavy flooding in the spring of 1974 and germination did not occur until flooding had subsided. Population 55 is upstream from 58,

and so would most likely be older and with *X. chinense* having a determinate growth system, the populations would have juvenile periods of different lengths. This may contribute to the differences in character means between these 2 populations. In population 59 (*X. pennsylvanicum*) the means for most characters are higher than for population 58 (*X. chinense*) despite the higher density, the later germination and the earlier flowering of this population. No doubt this is in part due to the good growth conditions of site 59, but it also suggests that *X. pennsylvanicum* has a faster growth rate than *X. chinense*. This is borne out by the smaller internode lengths of *X. pennsylvanicum* plants (smaller means for the number of major leaves). Experiments described in Chapter 5 look in more details at this,

The reproductive output (or fecundity) estimated for the 1974/75 season populations are shown in Table 4.13. The question is whether fecundity increases with density and if it reaches a maximum at some density. Reproductive output is determined not only by density but by the growth conditions which are difficult to quantify in the field. Thus at a fixed density there can be an increase in reproductive rate with improving growth conditions up to some optimal level. Table 4.13 gives some indication of the magnitude of the variation in fecundity possible. If all other factors were equal, then a comparison of population 58 with 55 would suggest that maximum fecundity for *X. chinense* would be obtained at a density between 33 and 102 plants/square metre. But the low fecundity figure for 58 is a result of the dichotomous age structure of the population, in which the small plants have none or very few fruit. Based on a conservative estimate of the size of the Mildura population site (500 square metres), the reproductive output of the region for the 1974/75 season is of the order of 2.7×10^6 fruit.

TABLE 4.13.

LEVELS OF REPRODUCTION IN 1974/74 SEASON FOR 5 POPULATIONS

Population	Reproductive Output Fruit/sq. metre
58	683
55	910
59	1345
56	480
57	177

Whether this is a realistic estimate of the seed available for the next season depends for a start on whether density and environmental factors have an effect on the percentage seed set. It also depends on the seed load or carry-over from previous seasons, and data presented earlier indicated that this could be quite large.

Do fruit always have a full complement of seeds? In Table 4.14. are listed, for several populations, the proportion of fruit which have 2 seeds, 1 seed and no seed. Thus populations 59 and 58 have high percentage seed set values and in fact for the populations, for which density is known, there is no correlation between density and seed set figures. On the other hand, the 3 *X. spinosum* populations which have strikingly low percentage seed set, were all from harsh dry sites with the plants small and single-stemmed, and having few fruits per plant. Almost all the *X. strumarium* populations examined, not only those in Table 4.14. but also those studied in the course of seed isozyme analysis have high seed set percentages (> 90%).

TABLE 4.14.

AMOUNT OF SEED SET IN SEVERAL POPULATIONS

Species	Population	% Fruit with 2 seeds	1 seed	0 seed
italicum	52	89	9	2
chinense	53	77	20	3
chinense	44	93	7	0
pennsylvanicum	59	92	4	4
chinense	58	95	5	0
spinosum	56	83	12	5
spinosum	57	74	24	2
spinosum	23	86	14	0
spinosum	27	60	35	5
spinosum	43	81	14	4
spinosum	31	73	22	5

The *X. spinosum* plants may initiate a number of flowers in which, if resources become limiting in the reproductive phase, not all seeds are developed. This is assuming that complete fertilization occurs and pollen production is not limiting. Low percentage seed set undoubtedly contributes to the unexpectedly large C.V. values for fruit weight for populations 53 and 57. It could be that a lot of the within family variation in seed weight (and hence fruit weight) is due to a combination of the determinate growth form of the plants and limited resources. In such a situation flowers at the apex would develop maximally into seeds; while as flowers ripen sequentially down the plant seeds become smaller or in the extreme case, do not develop at all. This means that the within family variability in populations could be environmentally induced and not necessarily genetic. This has not been tested in *Xanthium* but it has been shown to occur in several other species [reviewed by Harper *et al*, 1970].

An estimate of the initial seedling density in the Mildura population (59) can be made by an extrapolation of the figures shown on page 73 . The percentage germination taking upper and lower seeds together is 61% (after correcting for percentage seed set from Table 4.14) so the seedling density after emergence of the lower seeds and a fraction of the upper seeds is 168 plants per square metre. The fraction of the upper seeds (B seeds) which germinated was 25%. The population density, which was measured late in the reproductive phase, was 123 plants per square metre. If these two estimates of density are correct, there has been a self-thinning of the original seedling population by 26%, and hence there is evidence that at high densities *Xanthium* populations express a mortal response to density as well as a plastic one. Just what maximum seedling density is possible without self-thinning, and whether this differs between the races and the 2 species, is unknown. Observations of natural populations of *X. spinosum* suggest that the densities are rarely high enough to elicit a mortal response. Moreover, largely because the preponderance of habitats occupied by *X. spinosum* are disused paddocks and roadsides, it is subjected to more interspecific competition than *X. strumarium*. Only once has a population been observed in which the 2 species were randomly mixed and this was on a very small site adjacent to population 58.

It is unclear from the available data whether the plants that died were A or B plants. It would be expected that the B plants, if smaller, would be the first to be thinned as White and Harper [1970] have shown for other species. Undoubtedly, the seed population dynamics of *Xanthium* populations are more complex than has been presented here. Seed dormancy and germination are examined in detail, in the next

It is clear, however, that factors affecting seed dormancy

and germination can subsequently have an indirect effect on the expression of the phenotypic variation in the juvenile and reproductive phases of the life cycle.

In population 58 there is a mortal response to density, but it takes the form of genetic death, rather than biological death. Some of the plants survive to the end of the reproductive phase, using some of the resources, but they do not produce seed. Earlier it was shown that there was a bimodality in the structure of the population and that this originated in the differential germination of the upper and lower seeds of the fruit. Analysis of the distribution of the number of fruit per plant shows that 42% of the plants produced no fruit at all. Some 93% of these were B plants according to the classification based on plant height. This would suggest that the A plants, in this season at least, are the main contributors of seed to the next generation, with the fruit from the B plants making up a very small fraction of 1975/76 seed bank. Yet the A and B seeds are produced in equal numbers within the fruit generation after generation. This suggests that the seed is a fixed variation not determined genetically by the seeds themselves, but by the genes specifying the structure of the fruit.

Further studies are required to determine whether all races of *X. strumarium* and *X. spinosum* have similar plastic and mortal responses to density or whether there is variation between them in the amount and type of responses.

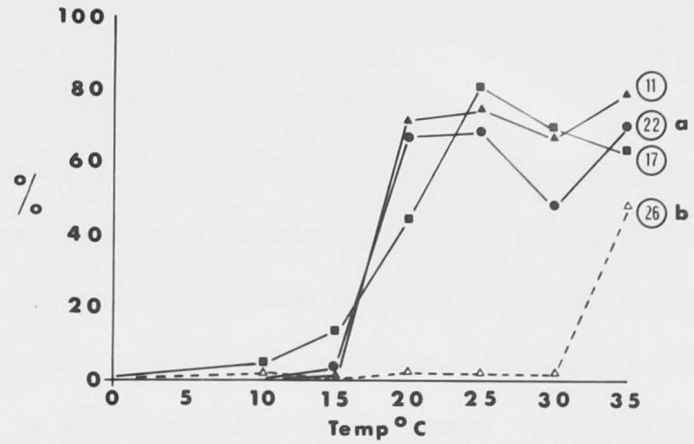


Fig.4.8(i) Effect of temperature on percentage germination of
a) X.chinense (pop. 11, 17, 22)
b) X.pennsylvanicum (pop.26)

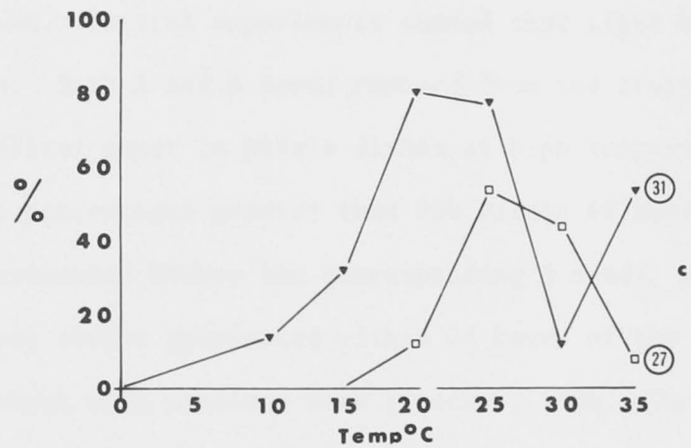


Fig.4.8(ii) Effect of temperature on percentage germination of
c) X. spinosum (pop.27, 31)

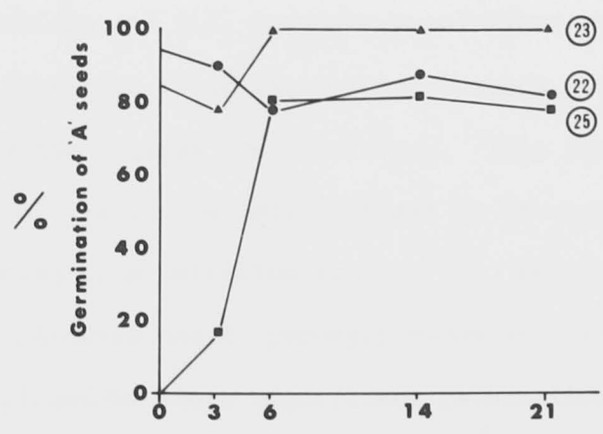


Fig. 4.9 Effect of the length of prechilling on percentage germination
X. spinosum (23), X.chinense (22),
X. pennsylvanicum (25)

4.3.2. Seed Population Dynamics

In Chapter 2, the literature relating to the seed dormancy of upper (B) seeds compared to lower (A) seeds in the fruit of *X. strumarium* was reviewed. There is scant published evidence of whether the same dormancy occurs in *X. spinosum*. Similarly, there are hardly any reports in the literature on the amount of quantitative variation in the germination responses of lower, as well as upper, intact seeds in the between populations of the same race, and between races of *X. strumarium*. Initial experiments showed that light had no effect on germination. Both A and B seeds *removed* from the fruit and placed on moistened filter paper in petrie dishes at high temperature (35°C) gave germination percentages greater than 90% within 48 hours. The A seeds normally germinated before the corresponding B seeds, although the latter almost always germinated within 24 hours of the A seeds. This is in agreement with previous work [Crocker, 1906; Thornton, 1935].

The effects of temperature on the percentage germination of seeds, when intact in the fruit, from 3 *X. chinense* populations, 1 *X. pennsylvanicum* and 2 *X. spinosum* populations are shown in Fig. 4.8. Overall less than 1% of the B (upper) seeds germinated and so in Fig. 4.8. only the A seeds are considered. This dormancy of the B seeds in intact fruit is in complete contrast to the normal germination of B seeds when removed from untreated fruit. The percentage germination of the races *X. chinense* and *X. pennsylvanicum* are very low below 20°C. In fact, *X. pennsylvanicum* shows significant germination only at 35°C. The figures suggest that overall for *X. strumarium* the optimum temperature for germination is 35°C. For the 2 *X. spinosum* populations the germination responses are more variable though the optimum temperature for germination is not 35°C for either population. The maximum germination for *X. spinosum* appears to be in the 20-30°C range and the results suggest that populations have

TABLE 4.15.

THE FRACTIONS OF THE POPULATIONS AT 10° AND 20° THAT (1) GERMINATED INITIALLY (G_1), (2) GERMINATED OVER THE REST OF THE SEASON (G_2), (3) GERMINATED FOR THE WHOLE SEASON (Gn) AND (4) DID NOT GERMINATE (R)

Species	Population		G_1			G_2			Gn			R		
			A	B	T	A	B	T	A	B	T	A	B	T
X. chinense	11	10°	17.3	0	8.7	12.7	0.7	6.7	30.0	0.7	15.3	70.0	99.3	84.7
		20°	39.3	0	19.7	6.0	1.3	3.6	45.3	1.3	23.3	54.7	98.7	76.7
X. chinense	36	10°	22.7	0	11.4	10.0	1.3	5.7	32.7	1.3	17.0	67.3	98.7	83.0
		20°	23.3	0	11.7	11.3	2.0	6.6	34.6	2.0	18.3	65.4	98.0	81.7
X. italicum	52	10°	17.3	0.6	9.0	13.3	2.7	8.0	30.6	2.7	16.6	69.4	97.3	83.4
		20°	7.3	0	3.7	14.7	0.6	7.6	22.0	0.6	11.3	78.0	99.4	88.7
X. pennsylvanicum	26	10°	9.3	0	4.7	32.7	4.6	18.7	42.0	4.6	23.3	58	95.4	76.7
		20°	1.3	0	0.6	35.3	1.3	18.3	36.6	1.3	18.9	63.4	98.7	81.1
X. spinosum	23	10°	90.6	0.6	45.6	0	16.7	8.3	90.6	17.3	53.9	9.4	82.7	46.1
		20°	29.3	0	14.7	29.3	5.3	17.3	58.6	5.3	31.9	41.4	94.7	68.1
X. spinosum	43	10°	23.3	0	11.7	50.6	7.3	29.0	73.9	7.3	40.6	26.1	92.7	59.4
		20°	35.3	0	17.7	32.6	3.3	18.0	67.9	3.3	35.6	32.1	96.7	64.4

different temperature optima for germination. The germination medium used in this experiment was soil but in the subsequent experiments well-moistened perlite was used since this gave higher germination responses and appeared to simulate more closely the situation in flooded river flats and disturbed cultivations. There was no evidence of differential interactions between germination medium and population.

The results of the main experiment are shown in Tables 4.15. and 4.16. In both (1) the first germination phase of the experiment (G_1), which is equivalent to the initial flush of germination at the beginning of summer, and (2) the second phase of germination (G_2) simulating germination in favourable field conditions over the rest of the season the percentage germination for *X. chinense* and *X. italicum* populations are very similar. Thus at 10, 20 and 30°C the 3 populations 11, 36 and 52 all have very low G_n figures for B seeds, while at 35°C the B seeds show 2% germination in the G_1 phase and in the region of 45% for the G_2 fraction in all 3 populations. Similarly, at 10°C and 35°C the G_n figures for the A seeds of the 2 races are in the region of 30% and 90% respectively with the G_1 and G_2 fractions being approximately the same for these 3 populations. However, at 20°C the G_n figure for *X. italicum* is lower due to a much smaller G_1 fraction whereas at 30°C the G_n fraction is not only smaller than those of *X. chinense* but its composition is different with 40% originating in the G_2 fraction, whilst almost all the G_n total for both *X. chinense* populations come from the G_1 fraction.

On the other hand *X. pennsylvanicum* exhibits a markedly different germination response, with almost none of the B seeds germinating even at 35°C. For the A seeds, the G_1 fraction was very small, except at 35°C, as was to be expected from previous results. The trend was

TABLE 4.16.

(a) THE FRACTIONS OF THE POPULATIONS AT 30°C AND 35°C THAT GERMINATED (1) INITIALLY (G_1), (2) OVER THE REST OF THE SEASON (G_2) AND (3) IN THE WHOLE SEASON (Gn)

Population			G_1			G_2			Gn		
			A	B	T	A	B	T	A	B	T
chinense	11	30°	84.0	1.3	42.7	3.3	10.0	6.7	87.3	11.3	49.3
		35°	81.3	2.0	41.7	11.3	45.3	28.3	92.6	47.3	69.9
chinense	36	30°	86.7	2.0	44.3	5.3	4.0	4.7	92.0	6.0	49.0
		35°	88.7	2.0	45.3	9.3	54.0	31.7	98.0	56.0	77.0
pennsylvanicum	26	30°	0.0	0.0	0.0	23.3	2.6	13.0	23.3	2.6	13.0
		35°	29.3	0.0	14.7	36.0	1.3	18.2	64.0	98.7	18.7
italicum	52	30°	42.7	0.0	21.3	30.7	10.7	20.7	73.4	10.7	42.1
		35°	88.0	2.0	45.0	2.7	40.7	21.6	90.7	42.7	66.7
spinosum	23	30°	86.7	1.3	43.7	4.0	5.3	4.6	90.7	6.8	48.8
		35°	50.0	0.6	25.3	0.6	0.0	0.3	50.6	6.0	28.3
spinosum	43	30°	48.0	0.0	24.0	1.3	1.3	1.3	49.3	1.3	25.3
		35°	27.3	0.0	13.7	2.0	0.0	1.0	29.3	0.0	14.7

TABLE 4.16.

(b) THE COMPONENTS OF THE NON-GERMINATING FRACTIONS (R) OF THE SEED POPULATIONS AT 30°C and 35°C; R = ED + ID + D, ED = ENFORCED DORMANCY, ID = INDUCED DORMANCY AND D = DEAD FRACTION

R			ED			ID			D		
A	B	T	A	B	T	A	B	T	A	B	T
12.7	88.7	50.7	3.9	72.0	38.0	0.0	4.1	2.0	8.8	12.6	10.7
7.4	52.7	30.5	2.0	14.0	8.0	1.4	25.4	13.4	4.0	13.3	8.7
8.0	94.0	51.0	0.0	76.0	38.0	4.0	2.7	3.4	4.0	15.3	9.7
2.0	44.0	23.0	0.0	8.7	4.3	0.7	16.0	8.3	1.3	19.3	10.3
76.7	97.4	87.0	20.5	19.3	20.0	43.0	69.0	56.0	13.0	9.1	11.8
64.0	98.7	18.7	15.6	21.3	18.4	39.1	50.1	48.1	9.3	27.2	11.8
26.6	89.3	57.8	10.0	50.0	30.0	13.6	17.6	15.5	3.0	17.3	10.2
9.3	57.3	33.2	1.3	27.4	14.3	3.4	16.6	10.0	4.6	13.3	8.9
9.3	93.2	51.2	-	-	-	-	-	-	4.7	28.7	16.7
49.4	94.0	71.7	-	-	-	-	-	-	12.6	44.7	28.7
50.7	98.7	74.7	-	-	-	-	-	-	21.3	28.7	25.0
70.7	10.0	85.3	-	-	-	-	-	-	22.0	36.0	27.0

reversed in the G fraction, such that over the temperature range, the G values were about the same. If this experiment does simulate the situation in the field during a season then it would appear that at low temperatures even with summer rains very little seed would germinate. The rate of germination for 2 of the races in the G_1 phase are very similar over the temperature range but the rates increase with increasing temperature (See Table 4.17). This is so for *X. spinosum* as well, except that the optimum rate is at 30°C rather than 35°C.

The only significant percentage germination of B seeds in either population of *X. spinosum* is at 10°C in the G_2 fraction. There is quite marked variation in the percentage germination of A seeds between populations 23 and 43 at 10, 30 and 35°C. This is undoubtedly, in part, due to the high percentage of empty seeds contained in the D fraction. On the basis of previous experiments, the high percentage of A seeds at 10°C was unexpected. The different optimum germination temperatures of *X. spinosum* populations indicate that there is considerable interpopulation quantitative variation in germination responses.

The components of R, the non-germinating fraction at 30°C and 35°C are shown in Table 4.16 (b). For the 2 *X. chinense* populations the biggest fraction of the A seeds is the D fraction and this is largely because of empty seed, while for the B seeds the ID fraction is nearly twice as large as the ED fraction at 35°C, but not at 30°C. In fact, if the combined G_n and ED fractions are compared at 30°C and 35°C, it can be seen that approximately 20% less germination occurs at 35° and this has gone into mainly the ID fraction, but also forms part of the non-viable component of the D fraction. This suggests that prolonged exposure of B seeds to 35°C induces dormancy in 10-20% of these seeds.

On the other hand for *X. italicum* the data suggests a lower ID fraction at 35°C but a higher fraction at 30°C compared to *X. chinense*.

For *X. pennsylvanicum* the major proportion of the non-germinating seeds is in the ID fraction. This seems to suggest that exposure to continual high temperatures leads to embryo dormancy in this race. The larger D fraction in this race especially, of the A seeds, is due to seed decay under the continual moist high temperature conditions of the experiment.

Since seeds cannot be removed without damage from the fruit of *X. spinosum*, the ED fraction could not be measured. The large D fractions in the *X. spinosum* populations was composed almost entirely of empty seed.

The aim of the next experiment was to determine if any of the species had a requirement for prechilling before germination and if the length of the prechilling period was critical. The results (see Fig. 4.9) demonstrate that the lower seeds of *X. spinosum* and *X. chinense* do not have any prechilling requirement for germination. However, after about 6 days or more prechilling there was a decisive increase in the percentage germination of *X. pennsylvanicum*. For prechilling periods of 6 days or more there was some increase in the percentage germination of B seeds from 0 to about 10% but only for *X. spinosum*.

In the second stratification experiment the effect of prechilling on the percentage and rate of germination of A and B seeds was examined in greater detail. In Table 4.18 the percentages in Phase 1 of the Table refer to germination in the first 2 weeks subsequent to the prechilling phase, while those in Phase 2 refer to the germination as a result of a further week of prechilling at 4°C after Phase 1. The percentage germination of the A seeds is very high for all populations. The percentage germination is as high for 26 as the other populations

TABLE 4.17

THE RATES OF GERMINATION$^{\phi}$ AT THE 4 TEMPERATURES IN THE G_1 PHASE OF THE EXPERIMENT

Species	10°	20°	30°	35°
chinense	.06	.10	.20	.33
italicum	.06	.11	.20	.25
spinosum	.07	.10	.20	.13

$^{\phi}$ Rate of germination based on time taken for 50% of the A seeds to germinate

indicating that *X. pennsylvanicum* does have a prechilling requirement for effective germination. The rates of germination for lower seeds in all populations are remarkably uniform (Table 4.18). There is in fact a flush of germination around the 4-6 day period with very little germination before or after this period. So it appears that under these special environmental conditions the quantitative variation in germination responses of the A seeds between all populations is very small.

However, the marked incidence of twins (simultaneous germination of A and B seeds of a fruit) in *X. spinosum* is in sharp contrast to almost no germination of the B seeds in the *X. pennsylvanicum* and *X. chinense* populations. The effect of the extra chilling period was that a small fraction of the B seeds germinated in all populations, except 38. Overall nearly a third of the B seeds in *X. spinosum* populations have germinated compared to less than 10% of the B seeds of *X. strumarium*. Since in this experiment the number and size of replicates is the same as in the main experiment a direct comparison of the germination percentages in Phase 1 with those in the G_1 phase

TABLE 4.18

THE EFFECT OF (1) 2 WEEKS PRECHILLING [PHASE I] AND (2) A FURTHER WEEK OF PRECHILLING [PHASE II] ON THE PERCENTAGE GERMINATION OF A AND B SEEDS

Population		Phase I			Phase II			Overall			Rate of germination of A seeds
		A	B	T	A	B	T	A	B	T	
chinense	11	93.0	1.3	47.2	2.7	7.6	5.2	95.7	8.9	52.3	.25
	38	100.0	0.0	50.0	0.0	0.0	0.0	100.0	0.0	50.0	.25
pennsylvanicum	26	93.1	1.3	47.2	0.7	9.7	5.2	93.8	11.0	52.8	.25
spinosum	23	92.8	22.7	57.8	0.7	5.0	2.9	93.5	27.7	60.6	.25
	30	97.1	28.2	62.7	0.0	6.5	3.3	97.1	34.7	65.9	.25

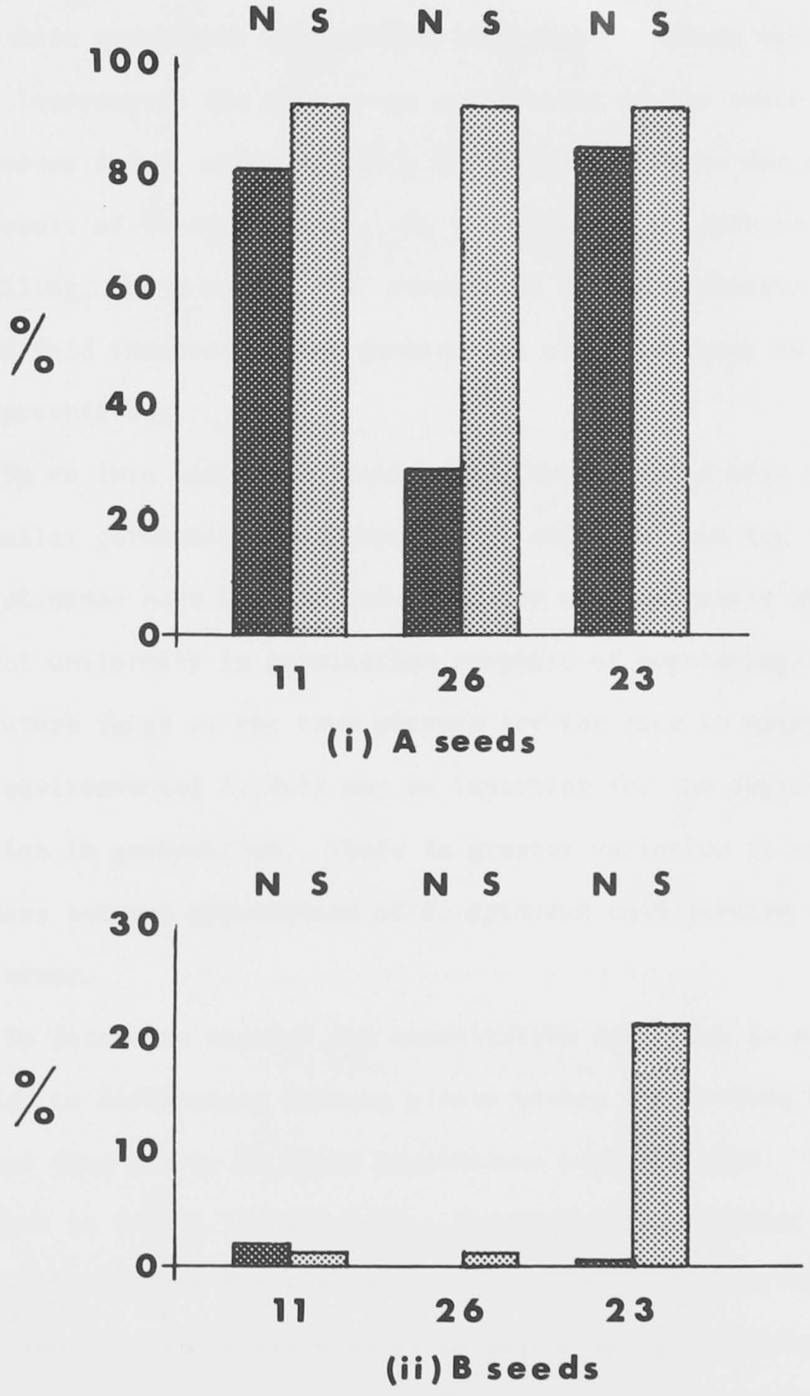


Fig. 4.10 (i, ii) Comparison of percentage germination of seeds from three populations with (S) and without (N) stratification.

of the main experiment can be made (Fig. 4.10). There was only a slight increase in the percentage germination of the lower seeds for *X. chinense* and *X. spinosum* but a three-fold increase for *X. pennsylvanicum* as a result of stratification. On the other hand, with or without prechilling, the B seeds of *X. strumarium* do not germinate, yet there is a 20 fold increase in the germination of the B seeds of *X. spinosum* after prechilling.

Up to this point the results have demonstrated that *X. italicum* has similar germination responses to *X. chinense*, but too few populations of *X. chinense* have been looked at to say with certainty whether the apparent uniformity in germination response of populations over the temperature range is the true picture for the race in Australia or not. Other environmental factors may be important for the expression of variation in germination. There is greater variation in germination responses between populations of *X. spinosum* than between those of *X. chinense*.

To determine whether the quantitative variation in germination extended to differences between plants within populations, half-sib families from plants in field populations were analysed. The results are given in Figs 4.11. and 4.12. The percentage germination figures have been corrected for the amount of seed set. Within the 3 *X. chinense* populations there are differences in percentage germination between plants, especially in 22 and 42. The mean percentage germination figures for these populations are lower than those obtained from random seed samples of other populations of *X. chinense*. Further work is required to determine whether this is a real difference or whether it would disappear of a large number of families were examined. Certainly population 13 appears to have a completely different germination response to other *X. chinense* populations. For these populations the variation

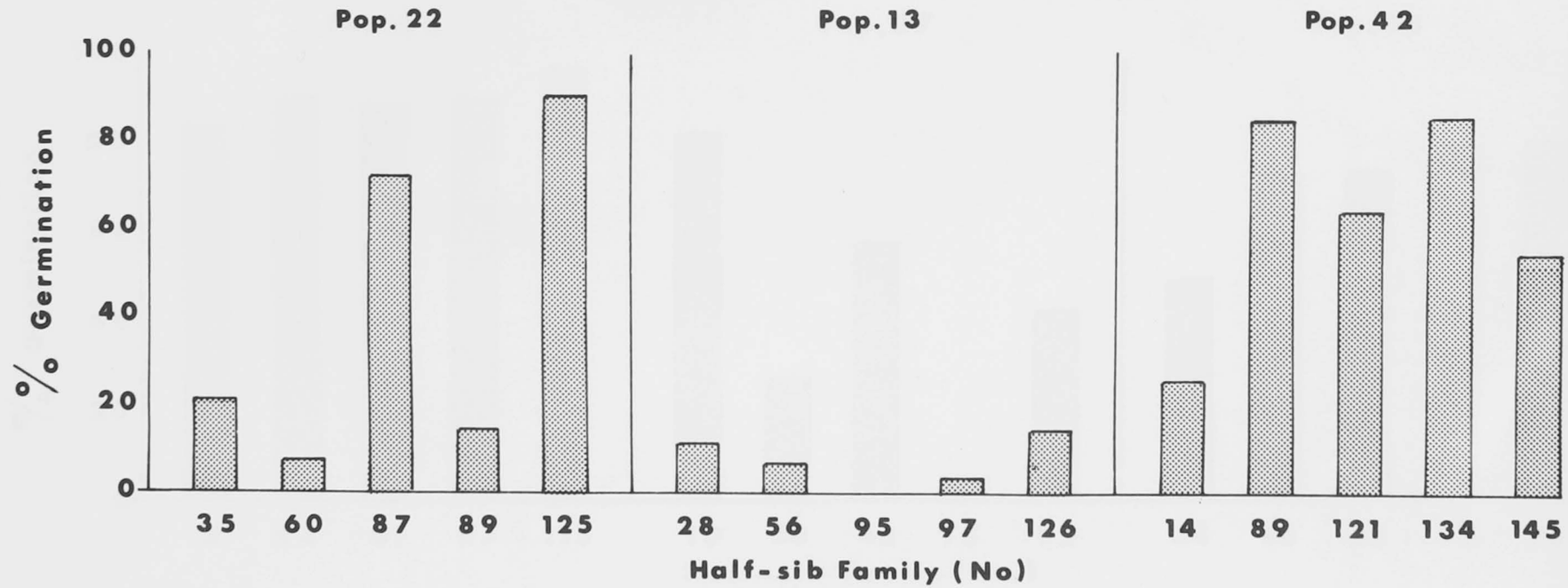


Fig.4.11 The percentage germination at 35°C of A seeds in half-sib families in three populations of X.chinense.

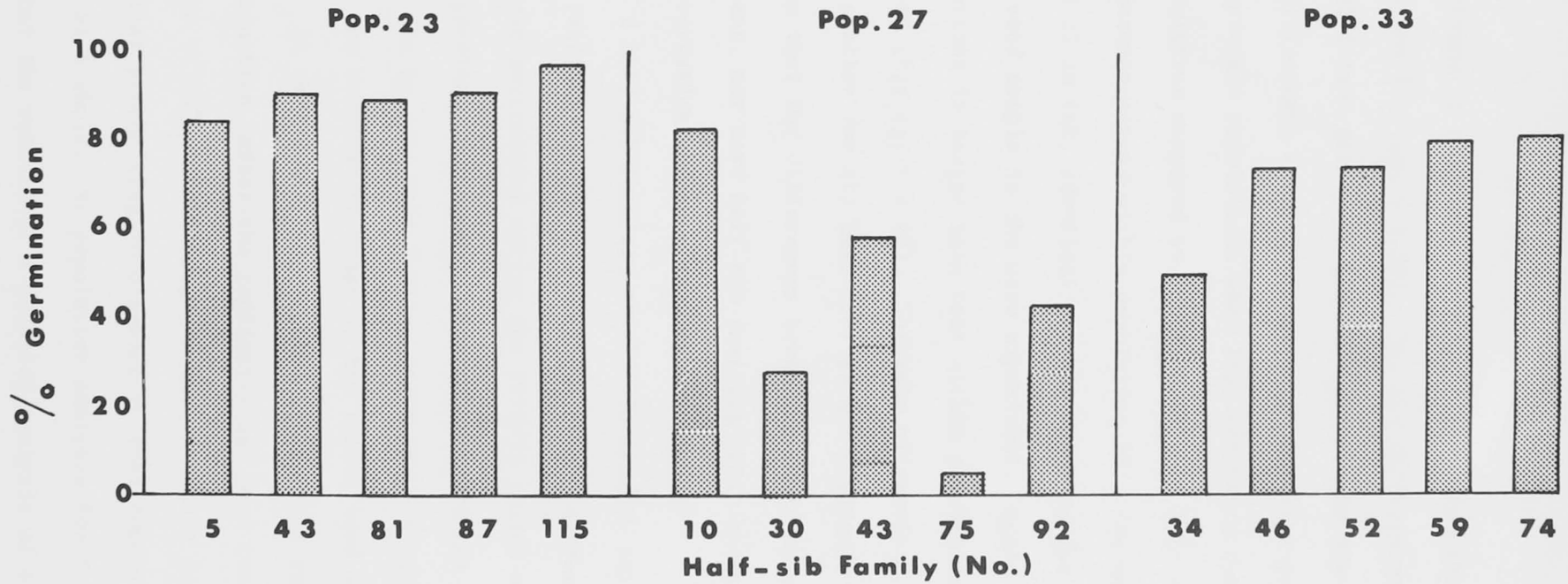


Fig.4.12 The percentage germination at 30°C of A seeds in half-sib families in three populations of X. spinosum.

between populations is not significantly greater than the variation within populations [$F_{(2,12)} = 1.33$]. So, the differences in germination behaviour of different plants in the same population are nearly as great as the differences between plants from different populations.

The *X. spinosum* populations show less variation both between and within population compared to *X. chinense*. In fact most of the variation is between plants within population 27. The mean percentage germination of 23 is 86%, identical to that obtained for this population from a random seed sample in the main experiment. Again the variation between populations is larger than that within populations, but not significantly so [$F_{(2,12)} = 1.67$]. The rate of germination (about .25) was very similar for all half-sibs in all populations. It was not possible to test for differences between seeds from different parts of the same plant, nor were half-sib families large enough to test within family variation across a temperature range.

However, 2 half-sib families,one(26/77) from *X. pennsylvanicum* and the other (44/135) from *X. cavanillesii* obtained from plants grown in the same field environment during the 1974/75 season were analysed in detail for germination responses over the temperature range. From Table 4.19. it can be seen that family, 26/79 shows a similar germination response, over the temperature range as the random seed sample from population 26. In this Table, the figures in brackets refer to the percentage germination, after the replicates had been transferred from that temperature to 35°C. These figures indicate that for family 26/79 there is a similar prechilling requirement to that demonstrated for the population as a whole. No population analysis for *X. cavanillesii* was possible, but the results of a detailed analysis of a single family are given in Table 4.19. These results suggest that this race behaves more like *X. spinosum* than the other races of *X. strumarium*. This

TABLE 4.19.

THE EFFECT OF TEMPERATURE ON THE PERCENTAGE GERMINATION OF A SEEDS OF 2 FULL-SIB FAMILIES

Family	10°	20°	30°	35°
26/79 *(pennsylvanicum)*	0 (70.9)	0 (8.4)	4.0	4.0
44/135 *(cavanillesii)*	0 (84.5)	0 (61.9	61.2	50.3

family has an optimum germination around 30°C rather than 35°C, and even exposure of fruit to only 10°C results in an enhancement of germination at 35°C by a third as much again.

For many species, seeds are dormant immediately after harvesting. A random collection of fruit from population 59 (*X. pennsylvanicum* - Mildura) harvested at the beginning of April was divided into 3 fractions, with 2 replicates of 50 fruit in each. One set was tested for germination at 35°C immediately, a second lot stored at 35°C for 2 weeks and then tested for germination and the other lot stratified at 4°C for 2 weeks and then tested at 35°C. There was no germination of B seeds at all and less than 3% for A seeds for any of the treatments. This confirms the work of Wareing and Foda [1957] who showed that both upper and lower seeds are dormant immediately after harvesting but the lower seeds gradually emerge from dormancy during a period of dry storage. There appears to be different types of dormancy in the seed of *Xanthium*. Whether the other races and *X. spinosum* have this post-harvest dormancy and whether after-ripening in dry storage will remove

it is not known. Crocker and Barton [1957] quote several examples of species in which freshly harvested seeds are dormant under some environmental conditions but not other. This could be the case in *Xanthium* but it has not been studied.

4.4. DISCUSSION

The aim of this part of the study was to measure and compare the quantitative variation in the species of *Xanthium*. The variation within the germination and adult phases of the life cycle was examined separately.

An analysis of the variation in several morphological characters in 10 field populations revealed that there are considerable amounts of quantitative variation in populations of *Xanthium*. The levels of variation differ between populations, and the variation appears to be bigger between families than within families. For the populations studied in this survey the amount of quantitative variation was greater overall for *X. italicum* compared to the other races of *X. strumarium* and *X. spinosum*. Lack of time precluded an analysis of field populations over the complete geographic ranges of the species nor the measurement of a larger number of morphological traits in the populations examined. Nevertheless the data demonstrated that in these inbreeding species the phenotypic variability in quantitative characters is as large or larger than that described for other inbreeding and colonizing species by previous workers [Allard *et al*, 1968; Jain, 1969]. But no conclusions can be drawn from this data about the genetic and environmental components of this quantitative variation. Field observations on density and other ecological variables presented in the results indicated that a considerable proportion of the inter and intra population variability could be environmentally determined. This

viewpoint is reinforced by the work of Wapshere [1974] who showed that with decreasing density there was an increase in size of several quantitative characters of *X. chinense*. Likewise, Kaul [1965, 1971] showed that 4 different seasonal forms of the *X. strumarium* race, which grow at different times of the year in India had different morphological characteristics in their own ecological niches. However, the differences between these forms in physiological and morphological characters disappeared to a large extent when grown under similar edaphic and moisture conditions suggesting once again that a lot of the observed variation in natural populations of *Xanthium* could be environmental in origin.

In Chapter 3, the results indicated that levels of qualitative genetic variation were unusually low and it appeared as if the population structure was one of remarkable genetic uniformity, with the exception perhaps of *X. italicum*. What genetic differences were present, were between races and species rather than within them. Previous work has shown that differences between races of *X. strumarium* in several quantitative characters were most likely genetic in origin [Love and Nadeau, 1961; McMillan, 1974b; Schull, 1934; Symons, 1926]. So the question that arises is whether all the genetic variation in *X. strumarium* is between races rather than within them and if *X. spinosum* is as genetically uniform as well. If so, it will be more like the situation described for inbreeders by Stebbins [1957], but contrary to what has been found in recent studies of inbreeders [Allard *et al*, 1968].

To confirm whether the genetic structure of *Xanthium* populations in Australia is one of marked genetic uniformity and to clarify what strategies make these species successful colonizers the environmental component of the quantitative variation was measured by monitoring the development flexibility of the same genetic material in several different environments. These experiments are the subject of the next chapter.

Harper *et al* [1970] listed *Xanthium* species in that group of plants having somatically controlled seed polymorphisms. The results from the germination experiments demonstrated that the difference in size between the A and B seeds is concomitant with a marked difference in germination behaviour of the 2 seeds. Previous work relating to this dormancy of the B seeds was reviewed in Chapter 2. There is a comparatively high temperature requirement for germination with optimum temperature for *X. strumarium* in the region of 35°C. This confirms previous results [Crocker, 1948; Schull, 1911; Thornton, 1935]. For *X. spinosum* the optimum temperature appears to vary between populations such that overall the interpopulation variability is greater in *X. spinosum* than in *X. strumarium*. Also *X. spinosum* populations have a higher percentage germination over a broader temperature range.

Taking temperature regimes as different environments, it can be seen that there is considerable variation in germination between environments. The results from experiments, in which random seed samples were used, indicate that generally all the populations of *X. chinense* and the one of *X. italicum* have very similar patterns of variation in germination behaviour for both types of seed over the environments, including those environments that have a prechilling phase. A similar stability of germination responses between populations has been shown for some weedy species of the Caryophyllaceae [Thompson, 1973]. However, *X. italicum* differs more from the *X. chinense* populations than the latter do between themselves.

On the other hand *X. pennsylvanicum* showed very little germination in these same environments, except when a prechilling phase was included. What has not been established is whether prechilled *X. pennsylvanicum* seed shows the same variation pattern over the whole environmental range as the other 2 races. It is suspected that this difference

A seeds of *X. pennsylvanicum* reflects a genetic difference between this race and the other 2 races and it could well be determined by a genetic component of the seed embryo rather than just the maternal genotype specifying the fruit structure. Because of the limited seed material little information could be gathered about the quantitative variation in germination responses for *X. cavanillesii*. If the results from the full-sib analysis of this race are indicative of the population as a whole then it shows a variation pattern over the environments somewhat different to that of the other races. There is less variation in germination between environments and higher overall germination but only in the same restricted temperature range.

Mann [1965] found that for *X. chinense* burrs planted in the field during September, 95% germinated during the summer and of these burrs 34% produced a second seedling in the same season. Similarly, Butler [1951] showed for Riverina plants of *X. spinosum* there was 84% germination of A seeds 3 weeks after sowing and this had increased to 91% by the end of the season. Both sets of data are in reasonable agreement with those of the main experiment and this indicates that in a simplified way the main experiment simulated the germination responses of *Xanthium* species over a season. Other factors such as depth of seed burial, flooding, fungal infection and predation have been shown to have a significant effect on regulation of seed numbers and germination responses [Mann, 1965; Wapshere, 1974; Kaul, 1965; Butler, 1951]. Field observations have indicated that there can be heavy insect predation of seed of *X. spinosum*, especially in some northern populations.

The main experiment showed that the amount of carryover of seed (seed load) to the subsequent seasons varies between environments. For example, number of both A and B seeds of *X. chinense* in the seed

load decrease with increasing environmental temperature, but at 35°C the proportion of B seeds of the seed load in the induced dormancy fraction had increased compared to the proportion at 30°C at least. What this work has not shown is the interaction of seed load, seed viability and decay, predation and other factors over and between seasons. Field studies like those of Sraukhan [1974] are required in which regular populations are monitored over several years.

There appears to be different types of dormancy in *Xanthium*. There is the classical dormancy of the B seeds in intact burrs, which is absent if initially seeds are removed from the fruit. The dormancy of A and B seeds in freshly harvested fruit at least of *X. strumarium* and of the A seeds of ripe *X. pennsylvanicum* have been demonstrated. As well there seems to be a high temperature embryo induced dormancy in *X. chinense* and *X. italicum* for a fraction of the B seeds. Thornton [1935] showed that embryo induced dormancy could be overcome by moist prechilling for 3 months.

The classical dormant condition has been attributed to impermeability of the testa to oxygen [Crocker, 1906; Davis, 1930], to the presence of inhibitors [Wareing and Foda, 1957] and to an inability of the B embryo to generate enough physical thrust to rupture the testa [Esashi and Leopold, 1968]. Only the inhibitor hypothesis requires a genetic component of the embryo to be involved in germination. However, Porter and Wareing, in an attempt to reconcile the different theories, suggested that inhibitors are probably the cause of the dormancy of the freshly harvested seeds. This could also be the cause of the A seed dormancy in *X. pennsylvanicum*.

Thus the dormancy of the B seeds is probably somatic, such that the quantitative variation in germination response between A and B seeds could be largely environmental. Nevertheless, this does not exclude

the possibility that differences within and between populations of one seed type could be genetically determined.

The data from half-sib analyses pointed to considerable variation in percentag germination between plants and between populations within a single environment. These results were somewhat at variance to those obtained from experiments using random seed samples. Cavers and Harper [1966] demonstrated similar variation in *Rumex* species. So in relation to the germination phase of life cycle there are indications of possible genetic differences between some races but not others and although there is considerable variation within races, how much, if any, is genetically determined is not known. For the few populations studied there was little evidence of geographic adaptation in germination responses though the results for *X. spinosum* are suggestive. The less critical germination requirements for *X. spinosum* no doubt partly explain its different geographic distribution and reports that it can grow at any time of the year [Everist, 1974; Parsons, 1974].

CHAPTER 5

PLASTICITY

5.1. PLASTICITY

In Chapter 3, the results indicated that there was a remarkable absence of allozyme variation within populations of *Xanthium* in Australia. Furthermore that data showed that both species are probably highly self-fertilized. As a result gametes will not come together completely at random at fertilization and there will be a reduction in genotypic types. The genetic flexibility for adaptation to new environments requires as a prerequisite, recombination to generate adaptive combinations different from those previously selected. Perhaps self-fertilizing annuals, such as the colonizing *Xanthium* species, in which recombination is restricted and allozyme variation is very low, have other colonizing strategies apart from adaptation to new environments by genetic variation. In Chapter 4, it was shown that there is considerable phenotypic variation for quantitative characters in populations of both species and at the same time the data strongly suggested that both species may have a strategy of high plasticity for these characters. As well, experiments on germination and dormancy demonstrated that there was considerable environmentally induced variation (plasticity) for these physiological processes.

Plasticity is the variation shown in a character by a genotype when its expression is altered by environmental conditions [Bradshaw, 1965]. Hence plasticity refers to all the environmentally induced variation shown by a genotype but does not include variation due to differences between genotypes. It is primarily manifested at the morphological and physiological levels, but most of the evidence for

plasticity has been concerned with morphological changes. The term stability is generally used to indicate a lack of plasticity for a particular character. Often lack of plasticity has been equated with homeostasis. Lerner [1954] argued that homeostasis, or uniformity in phenotypic expression in individuals of a given population was based on heterozygote advantage and that "established levels of obligate heterozygosity" are maintained within a species so that individuals are canalized into set phenotypic patterns. However, Lewontin [1957] defined homeostasis purely in terms of constancy of fitness, while Waddington [1957] used it in a somewhat broader sense. Because of this semantic confusion the term homeostasis will not be used in this study.

The plasticity of a species is not necessarily the same for all characters, but rather "the plasticity of a character is an independent property of that character, and is under its own specific genetic control" [Bradshaw, 1965]. Thus a species can be plastic for certain characters but exhibit stability in others. For many species reproductive characters with a high survival value, such as seed weight, have very low plasticities in marked contrast to many morphological characters associated with growth [Harper *et al*, 1970]. Moreover the plasticity of a character can vary between populations but is not necessarily correlated with similar plasticity changes in other characters. The plasticity of a particular character should be defined in reference to specific environmental conditions. There are numerous examples of environmentally induced variation in breeding systems, dispersal mechanisms, germination and photosynthetic mechanisms [Bradshaw, 1965; van der Pijl, 1972; Baker, 1972; Harper *et al*, 1970].

Plasticity may well occur at other levels of complexity such as at the biochemical level, where enzymes could have functional activity over a wider range of metabolic conditions than not only other enzymes in the plant but the same enzymes in closely related species [McNaughton, 1972]. There are some species, which for particular characters, have permanent phenotypic variation, but it is not strictly plastic in nature, since the variation is fixed independently of the environment. An example is somatically controlled seed polymorphisms [reviewed by Harper *et al*, 1970].

Typically annual weeds show very high overall plasticity in morphological characters, especially in response to density [e.g. Harper and Gajic, 1961; Baker, 1965; Bradshaw, 1965; Harper, 1967]. There are many other environmental variables, which can bring about plastic changes.

For annuals most of the plasticity of characters is between plant whereas in perennials it is within plants. Plasticity is used equally effectively by plants with indeterminate and determinate growth forms. Thus annuals with indeterminate growth systems in a short season under adverse conditions would have a low seed production, while in a long season seed numbers and other characters would be maximized. Plants with determinate growth forms are normally switched into this phase by some trigger, such as photoperiod, such that the levels of plasticity exhibited in the juvenile phase will be determined by environmental conditions.

Plasticity can be of an adaptive value to a plant species particularly for those characters influencing the amount of seed production. However, clearly in many situations, the plasticity of many characters is of a non-adaptive nature. Bradshaw [1965] reviewed in detail the adaptive value of plasticity and the types of selection

that can produce and maintain it. Examples of plants were given, in which the stability of certain characters was unrelated to the occurrence of heterozygosity although it was under genetic control. Direct experimental evidence of selection for plasticity is rare but changes in the level of stability of flower morphogenesis in wheat were obtained by selection [Frankel *et al*, 1969]. It could be envisaged that in varying environments genotypes with high plasticity would be selected for with the result that there would be evolution of plasticity as a mode of adaptation. For certain environmental factors, some species seem incapable of adaptation by a plastic response. It may be beyond the capacity of the species to achieve the necessary degree of adaptation by a system of plasticity. *X. strumarium* in cold regions of southern Australia where it is susceptible to frost before it can reproduce successfully. Perhaps, in some cases, plasticity acts as a buffer, so that selection pressures are avoided, although in response to extreme and sudden environmental changes a species would more likely adapt by means of genetic variation rather than plasticity.

What are the relative roles of genetic variability and plasticity as strategies of adaptation? It has been suggested that plasticity is maximized in autogamous annuals, yet in inbreeders the genetic variability is of the same magnitude as in outcrossing species [Allard *et al*, 1968; Allard and Kahler, 1971]. The "general purpose" genotype ascribed to many weeds by Baker [1965, 1974] is in effect one capable of plasticity in many characters and facets of its life cycle. Many colonizing species appear to have such genotypes, but for very few plants has it been demonstrated that a strategy of plasticity is critical for colonizing success to the exclusion of a strategy of genetic variation. Estimates of both plasticity and genetic variation within populations of species have rarely been made. Are they actually

opposing modes of adaptation with successful plant colonizers tending towards the extreme of high plasticity and concomitant low genetic variation; while high genetic variation is required for close permanent adaptation to more stable environments. Lewontin [1965] predicted that populations with low genetic variability for a character will be successful more often in irregular and unstable environments whereas those with high genetic variability will more often be successful in establishing colonies in stable environments. Levins [1963] on theoretical grounds suggested that there was an inverse relationship between plasticity and genetic variation such that in a comparison of 2 populations the more monomorphic would be expected to show greater plasticity. Perhaps then the more unstable the environment the greater will be the plasticity of the species occupying it.

There are very few plant species for which the environmental and genetic components of variation for several characters have been measured. Marshall and Jain [1968] looked at the relative roles of genetic variability and phenotypic plasticity in the 2 closely related colonizing *Avena* species. Both *Avena* species display greater plasticity in size and reproductive capacity in a wide range of environments. As was discussed previously Jain and Marshall [1967] showed that *A. barbata* is phenotypically more variable than *A. fatua*. To test whether *A. fatua* and *A. barbata* had different levels of phenotypic plasticity both species were grown in 24 diverse environments and the macroenvironmental differences for 10 morphological characters were used as a measure of environmentally-induced variation. The results demonstrated that the overall plasticity was greater in *A. barbata* than in *A. fatua*, while the genetic variation was higher in *A. fatua* than for *A. barbata*. This latter result agreed with earlier studies on qualitative

and quantitative morphological characters [Jain and Marshall, 1967] and was confirmed by later isozyme studies [Marshall and Allard, 1970a]. It was noticeable that these results were in agreement with Levins' [1963] predicted negative correlation between plasticity and genetic polymorphism.

In a study of the ecotypic variation in the plastic character, the leaf of *Ranunculus flammula*, which lives in more or less irregularly fluctuating environments, Cook and Johnson [1968] found that overall the most heterophyllous plants are the most adaptable and that these heterophyllous genotypes are best adapted for pioneering in new biotypes. They also showed that there was a loose correlation between the amount of plasticity in leaf shape (heterophylly) in a population and the stability of the environment. The level of development stability for number of flowers per head in 3 closely related *Liatris* species and their hybrids was examined by Levin [1970]. It was found that *Liatris cylindracea*, which exhibited the highest between-plant variance (genetic variation) also had the lowest within plant variance (plasticity) for this character.

Is there any relationship between the overall plasticity of species and the niche width and geographical ranges occupied by them? Unfortunately, for plants comparative estimates of plasticity for closely related species are meagre. Soulé and Stewart [1970] suggested that generalized broad niche species should be phenotypically and morphologically more variable than the more specialized and more narrow niched species (niche-variation hypothesis). However, the data relating to this and other "niche" hypotheses are very conflicting (van Valen, 1965; Pianka, 1974; Roughgarden, 1972]. In this discussion niche is being used to cover the total range of conditions

(including geographic range) under which the population of a species lives and replaces itself. Pianka [1974] has discussed at length the various definitions of a niche. For a plant, is this supposed higher variability of a broad niched species due to both plasticity and genetic variation? It would seem possible that plants occupying broader niches may do so because they have higher levels of plasticity compared to their relatives in narrower niches. Levins [1968] developed a model, which predicted that genetic heterozygosity per individual should increase with environmental variation. Bryant [1974] from an analysis of enzyme polymorphism data, from both poikilotherms and homeotherms claimed that about 70% of the geographic variation in heterozygosities could be related to specific variability in components of the environment.

Evidence suggests that plasticity as a strategy of adaptation is not necessarily common to all members of a genus and that in fact, such situations would be the exception rather than the rule [Baker, 1965]. In the genus *Xanthium* it is of interest to determine (i) whether the 2 species, and also the races of *X. strumarium* have the same levels of plasticity for individual characters and (ii) what is the relationship between the environmentally-induced variation and the genetic variation for such quantitative characters. The experiment described in this Chapter was carried out primarily to obtain estimates of variance components, which specifically measure the relative plasticities of the 4 races of *X. spinosum*. It should enable conclusions to be drawn about the nature of the considerable phenotypic variation in the field, which was documented in Chapter 4.

Fig. 5.1. The experimental design of the main experiment.

Environments	A		B		C		D		E	
Number of replicates/Env.	1	2	1	2	1	2	1	2	1	2
Species/Rep.	M	M	M	M	M	M	M	M	M	M
M = *X. strumarium*			N	N			N	N	N	N
N = *X. spinosum*										

Number of races/species	M				N
	I	II	III	IV	I
	X. chinense	*X. pennsylvanicum*	*X. italicum*	*X. cavanillesii*	*X. spinosum*
Number of populations/ race	11	25	46	46	23
	22	26			27
	42				43
Number of plants/Popn.	10	10	10	10	10
Number of plants/Rep./ Species	30	20	10	10	30

5.2. MATERIALS AND METHODS

5.2.1. Experimental Design

The experiment described in this Chapter was designed primarily to estimate the environmental and genetic components of variation of quantitative characters of *X. spinosum* and the races of *X. strumarium*. Hence, from the data of this experiment it was hoped the differences in relative plasticity and levels of genetic variation between species and races could be calculated and tested. Also with the use of several separate populations of *X. spinosum*, *X. chinense* and *X. pennsylvanicum* in this experiment the extent of genetic differentiation at the inter-population level can be determined. Moreover, the results should detail whether the species react to the different environmental regimes in the same or different manner as well as whether the different quantitative characters have the same levels of plasticity.

The design of the main experiment is shown in Fig. 5.1. The rationale underlying the experiment is that the same genetic material from each population is grown in the 5 different environments, so that the genetic and environmental components of variation are not confounded. The variation between environments is only environmental in origin, and thus is an estimate of the plasticity or environmentally-induced variation. On the other hand, variation within environments will largely be genetic in origin, though there will be some microenvironmental sources of variation within this component.

5.2.2. Environments

The 5 different environments were chosen so as to maximize the range of quantitative variation, and at the same time simulate some of the environments encountered by the species in their ecological habitats. The 5 environments were called A, B, C, D, E and for each

population there were 2 replications per environment. Details of the environments are as follows. Environment A consisted of 3 growth cabinets, in which both photoperiod and temperature were controlled. The 2 replicate blocks were subdivided over equal, but random areas in the 3 cabinets. Plants were grown in 5 inch plastic pots using a 3:1 soil:sand mixture with fertilizer. For the first 7 weeks the environmental regime was 8 hours dark at 30°C/16 hours light at 20°C per day. The photoperiod was then changed to a 12 hours dark/12 hours light cycle with the temperature regime unchanged and these conditions were maintained till harvest. Normal strength nutrient solution (aquasol) was applied in excess to the plants after 5, 6 and 8 weeks from the start of the experiment.

Environments B and C were separate glasshouses. In Environment B, a small glasshouse, the positions of the replicate blocks were randomly assigned with each replicate consisting of a block of 20 x 5 plants. Plants were grown in 5 inch pots of sand and nutrient solution was given in excess at the end of the fifth, seventh, ninth and eleventh weeks of the experiment. In Environment C, each replicate was a block of 7 x 10 plants with plants set up in 5 inch pots using the same 3:1 soil:sand mixture with fertilizer as used in Environment A. Nutrient treatments for plants in Environment C were the same as those of Environment B. In Environments A, B and C plants were watered twice daily.

Environments D and E were field plots with replicate blocks randomized within environments. Each replicate occupied 10 m^2 in area with plants in a fixed 10 x 10 array such that there was a fixed plant density of 1 per square metre. Plants in Environment D were grown under natural conditions with no supplementary fertilizer or watering. Environment E was the same as D for plant density and size

and arrangement of replicate blocks but plants in Environment E were irrigated two times per week and NPK fertilizer had been added to the soil at 500 lbs per acre prior to the start of the experiment. Also nutrient solution was added individually to the plants in Environment E in the sixth and tenth weeks of the experiment.

5.2.3. Populations

As shown in Fig. 5.1., 10 populations were used, details of which have been given previously in Table 2.1. Where possible, populations were chosen to enable comparisons to be made of variance components over the geographical ranges of races or species. Thus there were 3 populations of *X. chinense* (11, 22, 42), 3 of *X. spinosum* (23, 27, 43), 2 of *X. pennsylvanicum* (25, 26) and 1 each of *X. cavanillesii* (44) and *X. italicum* (46). From each population collection 10 half-sib families were chosen at random and from each half-sib family 2 plants were placed in each environment - 1 in each replicate. Only plants from A seeds were used in the main experiment. Seeds were germinated either on moist filter paper or in perlite, and transferred to peat pots the day after germination. Seedlings were transplanted into the environments when they were about 2 weeks old; this being in mid-November, 1974. Because of establishment problems *X. spinosum* was only placed in 3 environments, namely B, D, and E. Therefore the size of replicates in these environments was 100 plants while in A and C it was 70 plants.

A subsidiary experiment was also carried out to test whether the 2 seeds of a fruit (A and B seeds) were genetically the same, at least for some quantitative characters of the subsequent plants in 2 environments. The corresponding B plants to the A plants planted in the Environments B and E were also planted in these environments with

TABLE 5.1.

LIST OF QUANTITATIVE CHARACTERS SCORED IN THE EXPERIMENT

	Character	Time
1	Basal diameter of main stem (mm)	H
2	Plant height (cm)	H
3	Number of male flower clusters	P
4	Number of branches	H
5	Leaf width (mm)	P
6	Leaf length (mm)	P
7	Number of minor leaves	P
8	Number of major leaves	P
9	Number of fruits	H
10	Plant dry weight (g)	H
11	Days to flowering	
12	Mean fruit length (mm)	H
13	Mean fruit weight (mg)	H
14	Stem colour	F
15	Anther colour	At anthesis
16	Time of anthesis	
17	Maximum internode distance (mm)	H

H = harvest time, P = preharvest but post flowering
F = preflowering.

2 replicates per environment. The B block replicates contained 70 plants each, for this was done only for *X. strumarium* since the second seed could not be removed intact from *X. spinosum* fruit. Quantitative variation due to differential age structures within a population as a result of the dormancy of the B seeds is a phenomenon that has been observed in the field (Chapter 4). This source of variation was eliminated in this experiment and so it was possible to determine if the "twin" plants from a fruit have the same plasticity and genetic variance for these 2 environments.

5.2.4. Quantitative Characters and Analysis of Data

The 17 quantitative characters recorded, along with phase in the life cycle when they were measured are listed in Table 5.1. Branches were defined as vegetative shoots (excluding the main stem) greater than 6 cm in length. Leaf width and leaf length were of the largest leaf; the latter being determined by the leaf length (excluding the petiole). Minor leaves develop after the onset of the reproductive phase in the axils on the main stem and branches, and by this definition includes all leaves on vegetative shoots less than 6 cm in length. Plant dry weight included all of the plant above ground level. For each plant mean fruit length and mean fruit weight were estimated from a random sample of 5 fruits. Stem colour ranged from green through pink to red, and was scored on a scale 0 (green), 1 (pink) and 2 (red). Similarly, anther colour was black or yellow and these were scored as 1 and 2 respectively.

The basic data for any character consists of n_{ij} observations for the jth replicate under the ith environment. The nested analysis of variance for this data is shown in Table 5.2. [Kempthorne, 1957].

TABLE 5.2.

ANALYSIS OF VARIANCE FOR THE ESTIMATION OF VARIANCE COMPONENTS FOR THE MAIN EXPERIMENT

Source	d.f.	Mean square	Expected mean square
Between environments	E-1	M_E	$\sigma_G^2 + K_1\sigma_R^2 + K_2\sigma_E^2$
Between replicates within environments	E	M_R	$\sigma_G^2 + K_3\sigma_R^2$
Between plants within replicates	$\Sigma_{ij}(n_{ij}-1)$	M_G	σ_G^2
TOTAL	N..-1		

where N.. = total number of plants, $N_{i.}$ = number of plants in Env. i,

n_{ij} = number of plants in Env. i, Rep. j.

E = number of environments in which population was grown.

$$\hat{\sigma}_G^2 = M_G, \quad \hat{\sigma}_R^2 = \frac{1}{K_3}(M_R - M_G), \quad \hat{\sigma}_E^2 = \frac{1}{K_2}\left(M_E - M_G - \frac{K_1}{K_3}(M_R - M_G)\right)$$

$$K_1 = \frac{1}{E-1}\left\{\Sigma_{ij}\frac{n_{ij}^2}{N_{i.}} - \frac{1}{N..}\Sigma_{ij}n_{ij}^2\right\}$$

$$K_2 = \frac{1}{E-1}\left\{N.. - \frac{1}{N..}\Sigma_i N_{i.}^2\right\}$$

$$K_3 = \frac{1}{E}\left\{N.. - \Sigma_{ij}\frac{n_{ij}^2}{N_{i.}}\right\}$$

The between-environment variance component (σ_E^2) can be estimated and in a particular case is a measure of the variation of an individual character over those specific environments. The σ_R^2 term is an environmental term also, but in the present analysis is an experimental error due to differences between replicates within environments. The between plant (σ_G^2) variance contains the genetic component of variation though undoubtedly there are some microenvironmental sources of variation within this term as well.

The data of the subsidiary experiment were analysed in terms of a 3 factor Split-plot design with environments and seeds (A and B plants) being main plot factors and population a subplot factor. The results from the main experiment were also examined by a Split-plot 2 factor analysis of variance. Due to the marked difference between the 2 species for the characters fruit length, fruit weight, leaf length and leaf width the 2 species were analysed separately for these 4 characters. For the other 11 quantitative characters all 10 populations were used in the analyses. For the former analyses 'GENSTAT' was used, while for the latter both 'NO2FCT' and 'GENSTAT' programmes were used. These are statistical programmes available on the CSIRO computer. The Split-plot analyses were done on replicate means and this was possible since preliminary analyses done for each environment had shown that overall differences between replicates and the interaction of replicates and populations were not significant. Data for the 2 qualitative traits stem colour and anther colour were analysed by 2 χ^2 contingency table methods.

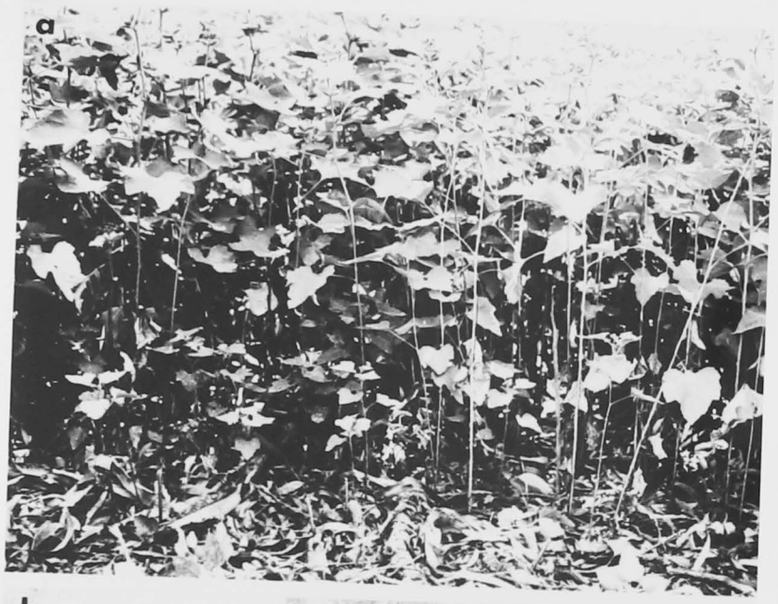
Where necessary, data for particular variables were transformed to a suitable scale by a $\log_{10}$ transformation.

Fig. 5.2. Replicates in 3 environments of the main experiment

(a) Environment B

(b) Environment C

(c) Environment D


a


b


c

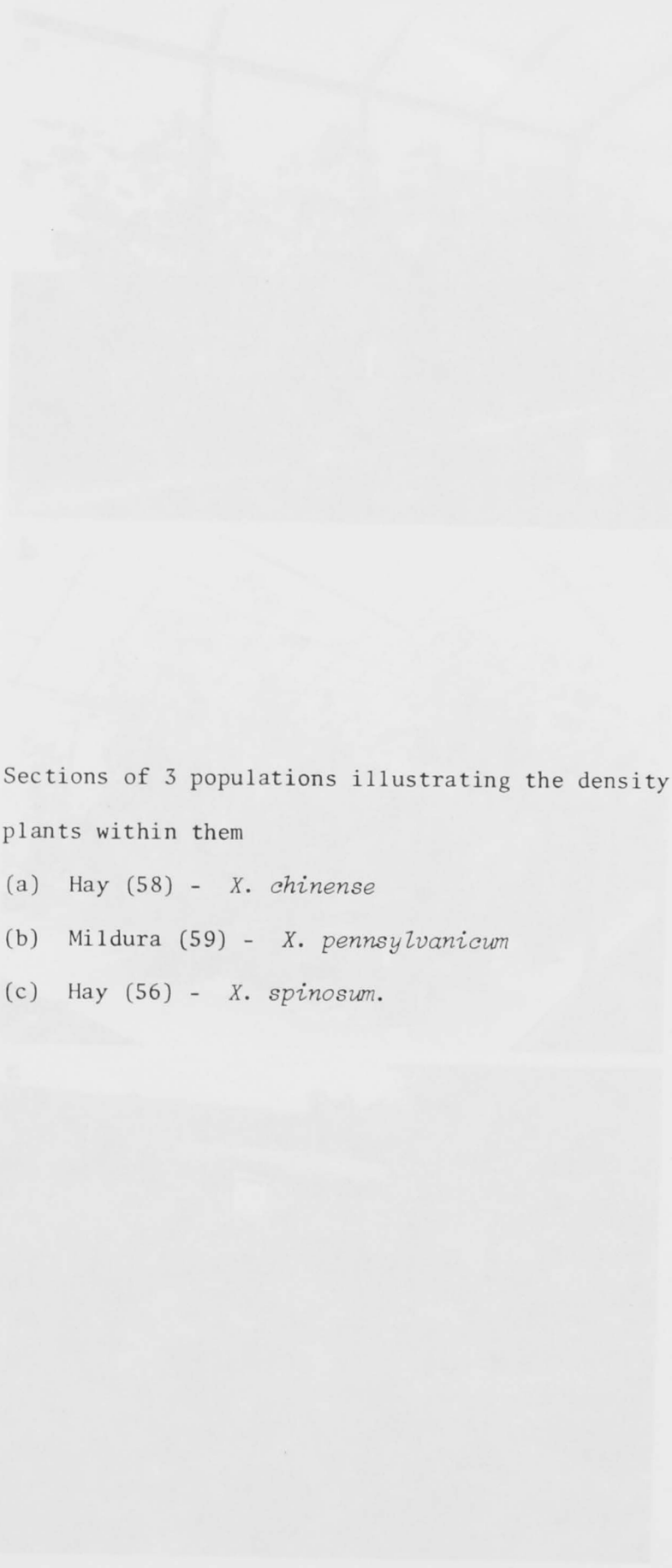


Fig. 4.4. Sections of 3 populations illustrating the density of plants within them

(a) Hay (58) - *X. chinense*

(b) Mildura (59) - *X. pennsylvanicum*

(c) Hay (56) - *X. spinosum*.


a


b


c

(e)

Fig. 5.3. Some of the individual plants in Environments B and E

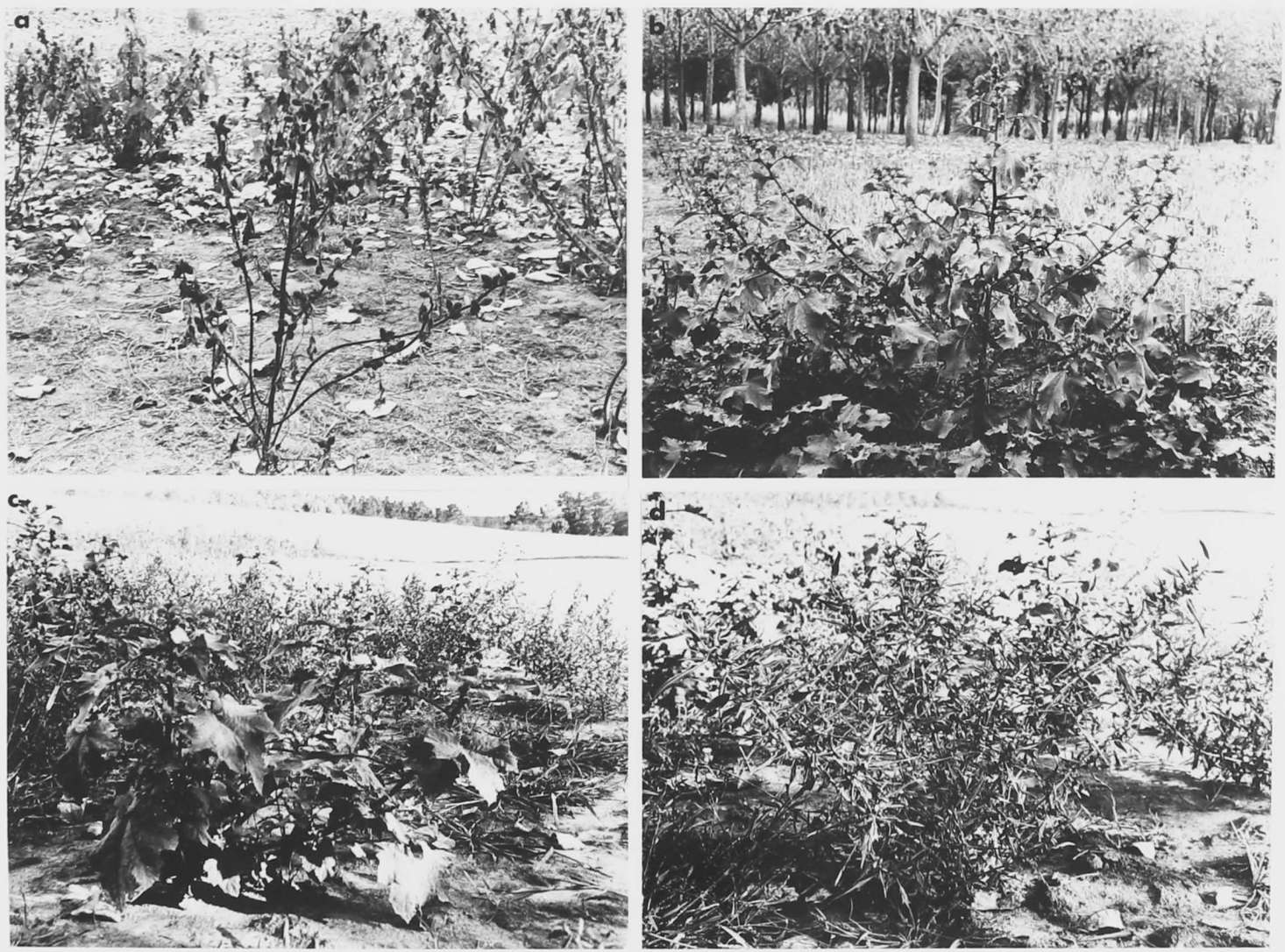
(a) *X. cavanillesii*

(b) *X. chinense*

(c) *X. italicum*

(d) *X. spinosum*

(e) Plants in Environment B.


a
b
c
d

5.3. RESULTS

The overall means and estimates of the 3 components of variance for each character and population of *X. strumarium* are shown in Tables 5.3, 5.4, 5,5 and 5.6. The corresponding data for each character for the 3 populations of *X. spinosum* are shown in Table 5.7. In Fig. 5.2, sections of 3 of the environments are shown. These show that plants in Environment B are single-stemmed and quite small, somewhat similar in appearance to plants growing under harsh field conditions. The plants in Environment C, however, although also in the main single-stemmed, or at least with a low number of branches, are very tall. Plants of this height are normally found in very favourable, but high density, situations in the field. Very large differences in the size of plants between environments were obtained as demonstrated by the photographs of individual plants from Environments B and E (Fig. 5.3.).

The means tabulated in Table 5.3. point to the genetically determined differences between races for some characters. For example, the days to flowering values reflect the differences in qualitative short day flowering requirement documented by McMillan [1975b]. Likewise fruit length and fruit weight means are a reflection of the differences in burr morphology of the races (see Fig. 2.4.). Probably the bigger maximum internode distances for *X. pennsylvanicum* and to a lesser extent *X. cavanillesii* and the concomitant smaller number of major leaves, but similar plant heights compared to the other races, are indicative of the faster growth rate for these 2 races. Similarly, the marked differences in leaf and burr morphology of *X. spinosum* and *X. strumarium* are reflected in the differences of the means of these characters as shown in Tables 5.7 and 5.3 (see also Fig. 5.4.).

TABLE 5.3.

OVERALL MEANS FOR EACH CHARACTER AND POPULATION OF *X. STRUMARIUM*

Character \ Population	*X. chinense*			*X. pennsylvanicum*		*X. cavanillesii*	*X. italicum*
	11	22	42	25	26	44	46
Basal diameter (mm)	11.7	12.3	12.1	9.5	10.6	9.4	10.9
Plant height (cm)	61.8	62.9	65.5	59.7	64.6	60.3	75.5
Number of clusters	41.8	48.0	48.1	31.9	44.9	18.6	51.4
Number of branches	3.8	4.7	4.7	2.9	4.2	1.7	5.7
Leaf width (mm)	139.5	146.2	147.6	109.3	113.1	110.0	119.6
Leaf length (mm)	125.2	131.5	135.1	106.9	113.4	110.7	114.5
Number of minor leaves	73.6	82.6	77.3	41.7	52.7	37.4	92.9
Number of major leaves	57.6	64.4	63.4	34.4	46.1	30.8	77.6
Number of fruit	82.9	105.2	109.7	49.7	79.7	26.6	82.6
Plant weight (g)	113.7	180.3	181.9	56.5	141.7	70.2	208.0
Days to flowering	104.0	100.4	102.3	63.1	61.4	82.1	101.3
Fruit length (mm)	18.5	18.9	18.4	21.1	21.2	22.6	20.3
Fruit weight (mg)	142.7	162.1	145.1	295.5	284.3	457.9	198.0
Time to anthesis	120.9	118.0	119.8	76.9	75.4	96.9	119.1
Internode distance (mm)	51.4	51.1	54.1	67.8	69.7	59.0	55.1

TABLE 5.4.

ESTIMATES OF THE ENVIRONMENTAL VARIANCE ($\hat{\sigma}_E^2$) FOR EACH CHARACTER AND POPULATION OF *X. STRUMARIUM*

Character \ Population	*X. chinense*			*X. pennsylvanicum*		*X. cavanillesii*	*X. italicum*
	11	22	42	25	26	44	46
Basal diameter	.048	.046	.040	.020	.036	.016	.065
Plant height	.034	.032	.022	.005	.010	.014	.023
Number of clusters	.273	.276	.228	.110	.187	.129	.450
Number of branches	.286	.258	.217	.139	.264	.105	.333
Leaf width *	834.73	855.79	701.14	246.49	156.41	304.88	777.97
Leaf length *	275.28	399.88	232.49	265.36	189.39	186.05	293.02
Number of minor leaves	.166	.172	.159	.020	.055	.023	.271
Number of major leaves	.145	.129	.100	.067	.132	.061	.181
Number of fruit	.456	.451	.396	.255	.355	.293	.643
Plant weight	.497	.469	.429	.234	.387	.246	.662
Days to flowering *	363.79	449.48	425.30	158.18	172.65	97.57	264.86
Fruit length *	2.72	2.21	2.87	1.08	1.17	.499	.402
Fruit weight	.007	.013	.010	.013	.015	.004	.004
Time to anthesis *	524.89	671.28	674.10	181.83	195.49	164.13	445.11
Maximum internode distance	.031	.028	.017	.012	.009	.022	.011

* Untransformed variables

Fig. 5.4. Some of the morphological characters in

(a) *X. pennsylvanicum*

(b) *X. spinosum*

a

b

The estimates of the between-environment variance ($\hat{\sigma}_E^2$) are overall much larger than the corresponding between-plant variance ($\hat{\sigma}_G^2$) and the between replicate variance ($\hat{\sigma}_R^2$) estimates. This is particularly so for *X. chinense*, *X. italicum* and *X. spinosum* populations. For both species the $\hat{\sigma}_R^2$ estimate for each character was in most cases either zero or very small and always considerably less than the corresponding $\hat{\sigma}_G^2$ terms. The exception was the *X. cavanillesii* $\hat{\sigma}_R^2$ values, which although smaller than the $\hat{\sigma}_G^2$ and $\hat{\sigma}_E^2$ values, were for several characters much larger than the $\hat{\sigma}_R^2$ values for all the other populations.

The F-max test [Sokal and Rohlf, 1969] was used to test whether the estimates of σ_E^2, σ_R^2 and σ_G^2 for each character were significantly different between populations within *X. chinense*, between populations within *X. pennsylvanicum* and also between the 3 populations of *X. spinosum* (for *X. pennsylvanicum* the F-max test reduced to the F test as there are only 2 *X. pennsylvanicum* populations). The more exact Bartlett's test for homogeneity of variance is somewhat unsatisfactory in this case because of the low degrees of freedom for the σ_E^2 and σ_R^2 components [Snedecor and Cochran, 1967]. From Table 5.8. it can be seen that many comparisons between σ_R^2 components were not possible since for many characters in at least 2 populations of a race the between replicate variance estimates were zero. There were no consistent significant differences between populations within *X. chinense*, *X. pennsylvanicum* or *X. spinosum* with respect to the σ_R^2 component of variance. For none of the characters was there significant interpopulation differentiation with regards to the between environment variance estimates for *X. chinense*, *X. pennsylvanicum* or *X. spinosum*. However, for most characters the σ_E^2 values were larger for population 26 compared to population 25, while those for population 42 were lower than for the other 2 populations of *X. chinense*.

TABLE 5.5.

ESTIMATES OF THE BETWEEN-PLANT ($\hat{\sigma}_G^2$) VARIANCE FOR EACH CHARACTER AND POPULATION OF *X. STRUMARIUM*

Character \ Population	*X. chinense*			*X. pennsylvanicum*		*X. cavanillesii*	*X. italicum*
	11	22	42	25	26	44	46
Basal diameter	.010	.011	.013	.011	.013	.015	.014
Plant height	.014	.014	.016	.023	.015	.018	.013
Number of clusters	.041	.049	.052	.069	.070	.070	.071
Number of branches	.036	.061	.054	.112	.079	.073	.079
Leaf width *	533.59	583.50	630.67	641.94	304.14	499.84	351.60
Leaf length *	296.90	317.14	399.67	479.37	273.85	253.02	253.57
Number of minor leaves	.035	.043	.058	.049	.030	.058	.057
Number of major leaves	.025	.040	.031	.049	.034	.043	.052
Number of fruit	.099	.080	.124	.125	.115	.109	.124
Plant weight	.076	.076	.106	.116	.107	.124	.097
Days to flowering *	6.93	11.81	11.78	29.00	9.56	43.269	21.97
Fruit length *	1.79	1.73	.810	1.42	1.36	4.93	2.04
Fruit weight	.012	.010	.008	.008	.008	.032	.019
Time to anthesis *	10.32	10.31	17.68	39.02	17.90	52.31	22.36
Maximum internode distance	.020	.018	.019	.015	.011	.013	.019

* Untransformed variable

TABLE 5.6.

ESTIMATES OF THE BETWEEN-REPLICATE ($\hat{\sigma}_R^2$) VARIANCE FOR EACH CHARACTER AND POPULATION OF *X. STRUMARIUM*

Character \ Population	*X. chinense*			*X. pennsylvanicum*		*X. cavanillesii*	*X. italicum*
	11	22	42	25	26	44	46
Basal diameter	.002	0	.004	.002	0	.005	0
Plant height	0	0	.001	.001	0	.006	0
Number of clusters	0	.005	.008	0	.006	.051	0
Number of branches	0	0	.009	0	0	.042	0
Leaf width *	64.9	60.4	18.7	0	42.3	10.05	5.39
Leaf length *	0	59.6	41.02	16.2	30.4	39.0	13.63
Number of minor leaves	.005	0	.014	0	.001	.021	0
Number of major leaves	.006	0	.017	0	0	.031	0
Number of fruit	.002	0	.010	.001	.010	.058	0
Plant weight	.002	.001	.019	.004	.002	.077	0
Days to flowering *	0	0	0	0	.332	0	0
Fruit length *	.035	0	.256	0	0	0	.331
Fruit weight	0	0	.001	0	0	0	0
Time to anthesis *	0	0	0	0	.259	0	0
Internode distance	0	.002	.001	0	.001	.002	.001

* Non-transformed variables

TABLE 5.7.

OVERALL MEANS AND ESTIMATES OF THE COMPONENTS OF VARIANCE FOR EACH CHARACTER AND POPULATION OF *X. SPINOSUM*

Character \ Population	Overall mean			$\hat{\sigma}_E^2$			$\hat{\sigma}_R^2$			$\hat{\sigma}_G^2$		
	23	27	43	23	27	43	23	27	43	23	27	43
Basal diameter (mm)	8.8	7.6	7.3	.071	.061	.064	.004	.004	.001	.027	.011	.013
Plant height (cm)	55.6	51.1	47.9	.037	.042	.037	.000	.000	.002	.029	.019	.021
Number of clusters	60.0	44.8	44.4	.462	.434	.387	.000	.000	.000	.100	.066	.110
Number of branches	7.5	5.7	5.6	.536	.517	.468	.000	.000	.001	.091	.054	.084
Leaf width (mm)*	21.7	23.0	19.7	4.73	11.84	18.10	.000	.000	3.32	28.30	15.99	12.42
Leaf length (mm)*	64.8	63.0	60.2	88.84	147.80	223.84	.000	.000	14.17	182.7	115.3	109.9
Number of minor leaves	208.5	161.1	165.2	.303	.291	.236	.000	.004	.000	.094	.053	.075
Number of major leaves	113.5	84.5	84.1	.360	.312	.337	.000	.011	.000	.074	.045	.060
Number of fruit	239.4	122.6	122.9	.783	.743	.607	.001	.007	.000	.206	.113	.133
Plant weight (g)	147.4	58.0	47.3	.939	.909	.800	.000	.005	.000	.185	.085	.172
Days to flowering *	89.3	92.0	91.3	89.54	75.17	101.14	.798	1.10	.000	17.68	11.97	9.16
Fruit length (mm)*	12.0	12.1	11.7	.363	.080	.394	.005	.166	.000	1.48	.498	.461
Fruit weight (mg)	78.4	73.5	70.0	.007	.009	.014	.000	.000	.000	.007	.004	.004
Time of anthesis*	108.3	113.0	108.8	47.52	83.06	110.6	1.00	2.52	1.46	26.52	14.80	33.05
Internode distance (mm)	32.3	32.4	34.1	.012	.019	.017	.000	.002	.000	.015	.015	.018

* Untransformed variables

On the other hand, of the F-max values for the between plant (σ_G^2) values 3 and 5 out of 15 are significant for *X. chinense* and *X. spinosum* respectively. The between plant variance (σ_G^2) values of the *X. pennsylvanicum* populations were significantly different for 6 out of the 15 characters. It was noticeable, however, that all 3 ratios for *X. chinense*, for 3 of the 5 for *X. spinosum* and 4 for *X. pennsylvanicum* were based on variances from untransformed variables. An initial screening of the original data had shown no multiplicative changes in means for these characters and it had appeared that transformations were not necessary. There was a trend for the estimates of the between plant (σ_G^2) variance to be larger for population 42 than for the other 2 *X. chinense* populations. Similarly for *X. spinosum* population 23 had larger estimates of between plant (σ_G^2) variance for 12 out of the 15 characters compared to the other 2 populations. For the majority of characters the estimates of σ_G^2 are larger for population 25 than for population 26.

The data indicated that there is some genetic differentiation with respect to the characters studied between populations within *X. chinense*, *X. pennsylvanicum* and *X. spinosum* and that this may be greater in *X. pennsylvanicum* and *X. xpinosum* than in *X. chinense*. On the whole the differences in components of variance estimates between races and species are much larger than the differences between populations within races. So to enable comparisons of between-environment and between-plant variances to be made between races and species, weighted average variances, for each character, and race were calculated where appropriate and these are listed in Table 5.9. It is acknowledged that because some σ_G^2 terms were significantly different between populations that this procedure is not rigorously correct.

TABLE 5.8.

F-MAX VALUES FROM COMPARISONS FOR EACH CHARACTER OF THE 3 VARIANCE COMPONENTS BETWEEN POPULATIONS WITHIN SPECIES

Character	*X. chinense*			*X. pennsylvanicum*			*X. italicum*		
	$\hat{\sigma}_E^2$	$\hat{\sigma}_R^2$	$\hat{\sigma}_G^2$	$\hat{\sigma}_E^2$	$\hat{\sigma}_R^2$	$\hat{\sigma}_G^2$	$\hat{\sigma}_E^2$	$\hat{\sigma}_R^2$	$\hat{\sigma}_G^2$
Basal diameter	1.20	2.00	1.18	1.80	-	1.18	1.16	4.00	2.45*
Height	1.55	-+	1.14	2.00	-	1.77*	1.14	-	1.53
Clusters	1.20	1.60	1.27	1.70	-	1.01	1.19	-	1.66
Number of branches	1.32	-	1.69	1.90	-	1.42	1.15	-	1.69
Leaf width	1.19	3.47	1.18	1.58	-	2.11**	3.83	-	2.28*
Leaf length	1.18	1.52	1.35	1.40	1.88	1.75*	2.52	-	1.66
Number of minor leaves	1.04	2.80	1.66	2.75	-	1.63*	1.28	-	1.77
Number of major leaves	1.45	2.83	1.60	1.97	-	1.44	1.15	-	1.64
Number of fruit	1.15	5.00	1.55	1.39	10.00*	1.09	1.29	1.17	1.82
Plant weight	1.16	19.00*	1.40	1.63	2.00	1.08	1.17	-	2.18*
Days to flowering	1.24	-	1.70*	1.09	-	3.03***	1.13	1.38	1.93
Fruit length	1.30	7.31	2.21**	1.08	-	1.04	4.93	33.2*	3.21**
Fruit weight	1.86	-	1.50	1.15	-	1.00	2.00	-	1.75
Time to anthesis	1.28	-	1.71*	1.08	-	2.18**	2.33	2.50	2.23*
Internode distance	1.82	2.00	1.05	1.33	-	1.36	1.58	-	1.20

+ Two between replicate variance estimates were zero.

*, ** - significant at the 5% and 1% probability level respectively.

TABLE 5.9.

ESTIMATES OF THE ENVIRONMENTAL AND GENETIC VARIANCES FOR EACH CHARACTER AND *XANTHIUM* SPECIES

Character	*X. spinosum**		*X. chinense**		*X. pennsylvanicum**		*X. cavanillesii*		*X. italicum*	
	$\hat{\sigma}_E^2$	$\hat{\sigma}_G^2$	$\hat{\sigma}_E^2$	$\hat{\sigma}_G^2$	$\hat{\sigma}_E^2$	$\hat{\sigma}_G^2$	$\hat{\sigma}_E^2$	$\hat{\sigma}_G^2$	$\hat{\sigma}_E^2$	$\hat{\sigma}_G^2$
Basal diameter	.065	.017	.045	.011	.028	.012	.016	.015	.065	.014
Plant height	.039	.023	.029	.015	.007	.019	.014	.018	.023	.013
Number of clusters	.428	.092	.259	.047	. 49	.070	.129	.070	.450	.071
Number of branches	.507	.076	.253	.050	.202	.096	.105	.073	.333	.079
Leaf width	11.56	18.90	797.22	582.58	201.45	473.04	304.88	499.84	777.97	351.60
Leaf length	78.88	136.0	302.55	337.90	227.38	376.61	186.05	253.02	293.02	253.57
Number of minor leaves	.277	.074	.166	.045	.038	.040	.023	.058	.271	.057
Number of major leaves	.366	.060	.125	.032	.100	.042	.061	.043	.181	.052
Number of fruit	.711	.151	.434	.074	.305	.120	.293	.109	.643	.124
Plant weight	.883	.147	.465	.051	.311	.112	.246	.121	.662	.097
Days to flowering	88.62	12.94	412.86	10.17	165.42	19.28	97.57	43.27	264.86	21.97
Fruit length	.279	.813	2.43	1.44	1.13	1.39	.499	4.93	.402	2.04
Fruit weight	.010	.005	.010	.010	.014	.008	.004	.032	.004	.019
Time to anthesis	80.39	24.79	623.42	12.77	188.66	19.50	164.13	52.31	445.11	22.36
Inter node distance	.016	.019	.025	.019	.011	.013	.022	.013	.011	.019

* weighted average variances - see text.

From Table 5.9 it can be observed that the estimates of the between-environment component are higher for *X. chinense* than the corresponding estimates for both *X. pennsylvanicum* and *X. cavanillesii* for all characters with one exception; the fruit weight value for *X. pennsylvanicum* is slightly larger than the value for *X. chinense*. As well 12 out of the 15 estimates are higher for *X. pennsylvanicum* compared to *X. cavanillesii*. On the other hand, overall *X. italicum* appears to have similar estimates to *X. chinense* for the between-environment variance component. Direct comparison of the σ_E^2 estimates for *X. spinosum* with the comparative values for the 4 races of *X. strumarium* is not rigorously valid, since *X. spinosum* was grown in only 3 environments and its environmental variance terms are based on only 2 degrees of freedom. To test whether the between-environment variance terms for each character were significantly different between races, pairwise comparisons were made using the F test (Table 5.10). F ratios for the untransformed variables, leaf width and leaf length were not calculated for between species comparisons, since the differences in variances unduly reflect the differences in means for these characters. The only comparisons that were significant were those involving the number of minor leaves of *X. cavanillesii* or in 1 case of *X. pennsylvanicum*. This lack of significance using the F test was undoubtedly due to the low degrees of freedom resulting from the limited number of environments possible under the experimental design. The F values emphasize that both the *X. italicum* and *X. chinense* environmental variance estimates are higher than the corresponding values for *X. pennsylvanicum* and *X. cavanillesii*. The magnitude of the F ratios from comparisons of *X. spinosum* σ_E^2 estimates with those of *X. chinense* and *X. cavanillesii* suggest that overall the between-environment variance is at least as high in *X. spinosum* as in *X. strumarium*.

TABLE 5.10.

F VALUES FROM PAIRWISE COMPARISONS OF BETWEEN-ENVIRONMENT VARIANCES (σ_E^2)

FOR EACH CHARACTER AND SPECIES

	ch ϕ *pen*	*ch*ϕ *it*	*ch* ϕ *cav*	*it* ϕ *pen*	*pen*ϕ *cav*	*it* ϕ *cav*	*sp*ϕ *ch*	*sp* ϕ *cav*
Basal diameter	1.61	1.44[a]	2.81	2.32	1.75	4.06	1.44	4.06
Plant height	4.14	1.26	2.07	3.29	2.00[a]	1.64	1.35	2.79
Number of clusters	1.74	1.74[a]	2.01	3.02	1.16	3.49	1.65	3.32
Number of branches	1.25	1.32[a]	2.41	1.65	1.92	3.17	2.00	4.83
Leaf width	3.96	1.03	2.62	3.86	1.51[a]	2.55	-	-
Leaf length	1.33	1.03	1.63	1.29	1.22	1.58	-	-
Number of minor leaves	4.37	1.63[a]	7.22*	7.13*	1.65	11.78*	1.69	12.04*
Number of major leaves	1.25	1.45[a]	2.05	1.81	1.64	2.97	2.93	6.00
Number of fruit	1.42	1.48[a]	1.48	2.11	1.04	2.20	1.64	1.48
Plant weight	1.50	1.42[a]	1.89	2.13	1.26	2.69	1.90	3.59
Days to flowering	2.50	1.56	4.23	1.60	1.70	2.72	4.66[a]	1.10[a]
Fruit length	2.15	6.05	4.87	2.81[a]	2.27	1.24[a]	8.71[a]	1.79[a]
Fruit weight	1.40[a]	2.50	2.50	3.50	3.50	1.00	1.00	2.50
Time to anthesis	3.30	1.41	3.80	2.36	1.15	2.71	7.76[a]	2.04
Internode distance	2.27	2.27	1.14	1.00	2.00[a]	2.00[a]	1.56[a]	1.38[a]

* Significant at 5% probability level

a Variance larger for denominator, all others with larger variances for numerator.

ϕ *ch*, *pen*, *it*, *cav*, *sp* stand for *chinense*, *pennsylvanicum*, *italicum*, *cavanillesii* and *spinosum* respectively.

TABLE 5.11.

F VALUES FROM PAIRWISE COMPARISONS OF BETWEEN-PLANT VARIANCES (σ_G^2) FOR EACH CHARACTER AND SPECIES

	pen[φ] / *ch*	*it* / *ch*	*cav* / *ch*	*it* / *pen*	*pen* / *cav*	*it* / *cav*	*sp* / *ch*	*sp* / *it*
Basal diameter	1.09	1.27	1.36	1.17	1.25	1.07[a]	1.55	1.21
Plant height	1.27	1.15[a]	1.20	1.46[a]	1.06	1.38[a]	1.53	1.77*
Number of clusters	1.49	1.51*	1.49	1.01	1.00	1.01	1.96**	1.30
Number of branches	1.92**	1.58*	1.46	1.22[a]	1.32	1.08	1.52	1.04[a]
Leaf width	1.23[a]	1.66*[a]	1.17[a]	1.35[a]	1.06[a]	1.42[a]	-	-
Leaf length	1.11	1.33[a]	1.34[a]	1.49[a]	1.49	1.01	-	1.30
Number of minor leaves	1.13[a]	1.27	1.29	1.43	1.45[a]	1.02[a]	1.64*	1.15
Number of major leaves	1.31	1.63*	1.34	1.24	1.00	1.21	1.88*	1.22
Number of fruit	1.62*	1.68*	1.47	1.03	1.10	1.14	2.04**	1.52
Plant weight	2.20**	1.90**	2.37***	1.15[a]	1.08[a]	1.25[a]	2.88***	1.69*[a]
Days to flowering	1.90**	2.16***	4.25***	1.14	2.24**[a]	1.97**[a]	1.27	2.51***[a]
Fruit length	1.04[a]	1.42	3.42***	2.42***	3.55***[a]	2.42***[a]	1.77*[a]	3.80[a]***
Fruit weight	1.25[a]	1.90**	3.20***	1.68*[a]	4.00***[a]	1.68*[a]	2.00**[a]	1.11
Time to anthesis	1.53*	1.75**	4.10***	2.34***[a]	2.68***[a]	2.34**[a]	1.94**	1.19[a]
Internode distance	1.46[a]	1.00	1.46[a]	1.46	1.00	1.46	1.19[a]	1.00

* - 5%
** - 1%
*** - .1%

a - variance larger for denominator, for all others numerator larger
ch, *pen*, *it*, *cav*, *sp* stand for *chinense*, *pennsylvanicum*, *italicum*, *cavanillesii* and *spinosum* respectively.

TABLE 5.12.

THE NON-PARAMETRIC 'SIGN' TEST-PROBABILITY LEVELS FROM PAIRWISE COMPARISONS OF ESTIMATES OF COMPONENTS OF VARIANCE FOR THE *XANTHIUM* SPECIES CONSIDERING ALL CHARACTERS SIMULTANEOUSLY

Between-plant variance (σ_G^2)	Between-environmental variance (σ_E^2)				
	chinense	*pennsylvanicum*	*italicum*	*cavanillesii*	*spinosum**
chinense	-	.001	1.000	.000	.039
pennsylvanicum	.302	-	.035	.035	.021
italicum	.035	.302	-	.007	.039
cavanillesii	.035	.302	1.000	-	.021
*spinosum**	.039	1.190	1.190	.039	-

* For comparisons involving *X. spinosum* only transformed variables were used

The non-parametric "sign" test [Sokal and Rohlf, 1969] for paired observations was used to test, when considering all characters simultaneously, which races and species were significantly different from each other in their environmental variance (σ_E^2) components. The probability levels obtained are tabulated in the right half of Table 5.12. They indicate the following:

(1) *X. chinense* has a significantly greater environmental variance (σ_E^2) than *X. pennsylvanicum* (P = .001) and *X. cavanillesii* (P = .000)

(2) *X. italicum* has a significantly greater environmental variance (σ_E^2) than *X. pennsylvanicum* (P = .035) and *X. cavanillesii* (P = .007)

(3) *X. italicum* has a similar environmental variance (σ_E^2) compared to *X. chinense* (p = 1.000).

(4) *X. pennsylvanicum* has a significantly greater environmental variance (σ_E^2) than *X. cavanillesii* (P = .035).

(5) *X. spinosum* has a higher environmental variance component than the 4 races of *X. strumarium*, but this should be qualified in that estimates for *X. spinosum* are probably overestimates.

Hence, at least in the environments used in this experiment, the levels of environmentally induced variation were lowest in *X. cavanillesii* *X. pennsylvanicum*. Considering the diversity of characters studied, the consistency of these differences in environmental variance between races was very significant, especially since actual plasticity levels for individual characters were in many cases very distinctive from one another.

Contrary to the results for between-environment (σ_E^2) components, the estimates of the between-plant (σ_G^2) variance were smaller for *X. chinense* for 10 out of the 15 characters compared to *X. pennsylvanicum* and for 12 out of 15 characters compared to *X. cavanillesii*. Nevertheless, the estimates of between-plant (σ_G^2) variance were larger for *X. italicum* for 12 out of the 15 characters compared to *X. chinense* (Table 5.9). *X. spinosum* appeared overall to have similar levels of between-plant variance to *X. italicum*. The F test was used to test whether the estimates of σ_G^2, for each character, were the same for *X. spinosum* and the 4 races of *X. strumarium*. The F values in Table 5.11 emphasize the lower genetic (between-plant) variances for *X. chinense* compared to both the other 3 races of *X. strumarium* and *X. spinosum*. The estimates for 5 characters are significantly greater for both *X. pennsylvanicum* and *X. cavanillesii* than for *X. chinense*, while 8 σ_G^2 estimates are significantly greater for *X. italicum* than for *X. chinense* at the 5% level. It was noticeable that overall *X. italicum*, *X. chinense* and *X. pennsylvanicum* had fairly similar levels of between-plant variance. If anything, though it was not in any way significant, the F values indicated that *X. cavanillesii* tended to have a higher between-plant (σ_G^2) variance than *X. pennsylvanicum* which in turn tended to have a higher estimate than *X. italicum*. For 7 out of the 15 characters the between-plant variance (σ_G^2) was significantly greater for *X. spinosum* than for *X. chinense*.

The non-parametric "sign" test was used to test whether the between-plant variances of the 4 races and *X. spinosum* are equivalent when considering all the characters simultaneously. The between-plant variance is largely genetic in origin, with the rest originating in microenvironmental sources of variation. The probability levels in Table 5.12 indicate that the between-plant variance for *X. chinense*

is significantly lower at the 5% level than those for *X. italicum*, *X. cavanillesii* and *X. spinosum*, but is not significant at the 5% level compared to *X. pennsylvanicum*. Thus, *X. chinense* has less quantitative genetic variation for the characters studied than *X. spinosum* and the other less widely distributed races. The non-parametric test did not detect differences between the other 3 races of *X. strumarium*. It did, however, indicate that overall *X. spinosum* had greater genetic variation for the quantitative characters than *X. strumarium*.

To examine further the relationships between plasticity, population differentiation and race and species differences Split-plot analyses of variance were carried out on the replicate means of the original data. Details of these analyses for each character are presented in Appendices D, E and F. The summaries of the significance levels of the F values, which are given in Table 5.13 and 5.14, show that for almost all characters there is a significant environment x population interaction. From the Tables of means for each character and population (Appendix H) it can be seen that for the majority of characters the means increased across the environments in the same direction. Moreover, for the majority of characters, populations within a race exhibited similar means in the 5 environments, while the ranges of means across environments for races were often significantly different. This is not surprising since differences between races in environmental variance have already been demonstrated. With the use of the LSD values very definite common trends emerged. Thus the means for *X. cavanillesii* and to a lesser extent for *X. pennsylvanicum* of basal diameter, number of clusters, number of branches, number of minor leaves, number of major leaves, number of fruit and plant weight are consistently less in Environments B, C, D and E than the corresponding

TABLE 5.13.

SUMMARY OF SIGNIFICANCE TESTS OF F RATIOS FROM SPLIT-PLOT 'ANALYSIS OF VARIANCE' OF CHARACTERS FOR BOTH SPECIES TOGETHER

Character	Env.	Popn.	Env. x Popn.
Basal diameter	***	***	***
Plant height	***	***	***
Number of male clusters	***	***	**
Number of branches	***	***	***
Number of minor leaves	***	***	***
Number of major leaves	***	***	**
Number of fruit	***	***	*
Plant weight	***	***	**
Days to flowering	***	***	***
Time of anthesis	***	***	***
Internode distance	***	***	***

*, **, *** - Significant at the 5%, 1% and 0.1% probability level respectively.

means for *X. chinense* and *X. italicum*. However, these differences were not present in Environment A, but 2 other differences in characters were present. Plant height and internode distance were significantly higher for *X. pennsylvanicum* compared to *X. chinense* and for both characters *X. cavanillesii* means were higher though not significantly compared to *X. chinense*. These results suggested that the developmental rate of growth was higher in *X. pennsylvanicum* and

TABLE 5.14.

SUMMARY OF SIGNIFICANT TESTS OF F RATIOS FROM SPLIT-PLOT ANALYSIS OF VARIANCE OF 4 CHARACTERS WITH THE 2 SPECIES ANALYSED SEPARATELY

Character	Species$^{\phi}$	Env.	Popn.	Env. x Popn.
Leaf width	ST	***	***	**
	SP	**	**	NS
Leaf length	ST	***	***	*
	SP	**	*	NS
Fruit length	ST	NS	***	***
	SP	NS	NS	NS
Fruit weight	ST	**	***	***
	SP	*	**	*

ϕ ST = *X. strumarium*
SP = *X. spinosum*

NS Not significant at 5% probability level

*, **, *** Significant at the 5%, 1% and 0.1% probability levels respectively.

also perhaps in *X. cavanillesii* than that of *X. chinense*, especially since the number of major leaves was also smaller for these 2 complexes.

The different qualitative short day requirements of the races was borne out by the differences in means for days to flowering between races. These generally confirm the critical night length determinations of McMillan [1975b]. The control of photoperiod as part of Environment A has resulted in very small differences between races for this character. In other environments the character is fairly constant for a particular

race as would be expected, though plasticity was detectable in the harshest Environment B. In Environment B, flowering is significantly delayed for *X. spinosum* and all races except *X. pennsylvanicum* compared to Environments C, D, E and this is probably due to poor edaphic conditions. On the other hand, *X. pennsylvanicum* exhibits a very early initiation of flowering in the very favourable soil, nutrient and moisture conditions of Environment C. The resultant short juvenile phase of the life cycle and concomitant early switch to a determinant growth form is reflected in the significantly lower mean plant height for *X. pennsylvanicum* compared to *X. chinense* and *X. italicum* in this environment. There was a slight trend evident within *X. chinense* of earlier flowering within population 22, and this may be an indication of adaptation to the lower latitudes. Certainly within population 42, one half-sib family consistently flowered significantly later in all 4 environments so much so that members of some replicates in Environments D and E were killed by frost before reaching anthesis.

Overall, there was also evidence of a trend of lower means for population 11 compared to the other 2 *X. chinense* populations. This could possibly reflect an adaptive plastic response to the shorter duration of the juvenile phase of the life cycle, in northern Queensland. The mean anthesis time for *X. chinense* was significantly longer in the field Environments D and E than in the glasshouse Environments B and C, despite the prolonged flowering dates in Environment B. This was also the case for *X. italicum* and was undoubtedly due to the onset of the very cool temperatures of autumn in Canberra. It had the result that fruit legnth and fruit weight means for *X. chinense* populations were significantly lower in the

TABLE 5.15.

COMPARISON OF THE MEAN NUMBER OF FRUIT PER PLANT FOR EACH SPECIES IN THE 5 ENVIRONMENTS

	Environment - mean number of fruit/plant				
	A	B	C	D	E
X. chinense	18	24	75	416	1233
X. italicum	12	17	52	423	1195
X. pennsylvanicum	24	15	46	154	657
X. cavanillesii	11	7	13	84	282
X. spinosum	-	17	-	256	1074

field environments. The differences in leaf shape in the 4 races were emphasized by the highly significant population F-ratios for leaf width and leaf length (Table 5.14). The range of means in leaf length was about the same for all populations though the leaves of *X. chinense* were actually the longest. Similarly in all environments *X. chinense* had the widest leaves and it, along with *X. italicum*, had a larger range than the other 2 races.

In Table 5.15, the mean number of fruit per plant for each race in each environment is tabulated. Although *X. italicum* had both a higher environmental variance and between plant variance for the number of fruit than *X. chinense* it has lower means in Environments A, B, C. In fact, in each of these 3 environments *X. italicum* has only 70% of the reproductive output of *X. chinense*. In the western areas of the *X. chinense* distribution such fruit productions per plant would make up the bulk of reproductive output in the majority of years. Large stands of plants of the average size in Environment E are rare for any of the races and even for *X. spinosum*.

As for *X. strumarium*, *X. spinosum* shows a similar range of means overall for most of the characters in Environments B, D and E. *X. spinosum* plants tended to have a small growth form in Environment B which was demonstrated by the significantly lower means for basal diameter, plant weight and plant height compared to *X. strumarium*, yet it did not have a significantly lower fruit production than the 4 races. Characteristic of the species, it tended to have more minor and major leaves than *X. strumarium* in all environments. Flowering was initiated sufficiently early in *X. spinosum* such that time to anthesis and fruit production was unaffected by the onset of cold weather. Mean fruit weight was not significantly different for the 3 populations in the field environments. However, for all 3 populations the fruit weights were significantly lower in Environment B, probably due to scarcity of nutrients, and the harsh environment conditions generally.

There were interpopulation differences within *X. spinosum*. For all environments, but particularly the field ones, the means for many of the characters of plants from population 23 were significantly higher than the corresponding means of population 43 and 27.

Stem and petiole colour were scored on a scale as previously detailed. The results are sumarized in Table 5.16 and 2 points were evident from this table. First, under the environmental conditions in the growth cabinets (Environment A) there were no differences in pigmentation between races, and second, in the other environments *X. chinense* had stems and petioles of a deep red colour and these were quite distinct from the other 3 races. In the field *X. chinense* has been observed with pure green pigmentation under very high density conditions so it is suspected that light is the factor influencing the expression of this character. *X. spinosum* showed no variation in stem

TABLE 5.16.

SUMMARY OF STEM AND PETIOLE COLOUR PIGMENTATION FOR EACH RACE IN EACH ENVIRONMENT

	ENVIRONMENT				
Race	A	B	C	D	E
X. chinense	GREEN	RED	RED	RED	RED
X. pennsylvanicum	GREEN	GREEN or PINK	GREEN or PINK	PINK	PINK
X. cavanillesii	GREEN	PINK	GREEN	PINK	PINK
X. italicum	GREEN	GREEN or PINK	GREEN	PINK	PINK

or petiole pigmentation. The anthers of *X. chinense* were black in colour, while those of the other 3 races were yellow. The only exception was a half-sib family of *X. italicum* in which most plants had black anthers. It would seem that this character could be under qualitative genetic control. The anthers of *X. spinosum* ranged in colour from brown to black but there were not discrete types.

In the subsidiary experiment the A and B plants from corresponding A and B seeds of a fruit were compared for their quantitative characters in Environments B and E. Details of the analyses are given in Appendix G, and a summary of the significance tests of F ratios for mean square terms involving seeds (A and B) are tabulated in Table 5.17. For 4 characters the means for the A plants were significantly higher in both environments for all populations than those of the B plants. There were no significant differences between A and B plants for the characters plant weight, days to flowering and time to anthesis. On the other hand, 5 characters had a significant environment x seeds interaction (Table 5.17) which was because they did not have different means in Environment B but the A plant means were significantly higher than the

TABLE 5.17.

SUMMARY OF SIGNIFICANCE TESTS OF F RATIOS FROM SPLIT-PLOT 'ANALYSIS OF VARIANCE' OF CHARACTERS FROM A AND B PLANTS

	Seeds	Env. x Seeds	Seeds x Popn.	Env. x Seeds x Popn.
Basal height	*	NS	NS	NS
Basal diameter	NS	NS	NS	NS
Clusters	*	*	NS	NS
Number of branches	**	**	NS	NS
Leaf width	*	**	**	**
Leaf length	*	**	NS	NS
Number of minor leaves	*	NS	NS	NS
Number of major leaves	*	NS	NS	NS
Number of fruit	*	*	NS	NS
Plant weight	NS	NS	NS	NS
Days to flowering	NS	NS	NS	NS
Time to anthesis	NS	NS	NS	NS
Fruit length	NS	NS	*	NS
Fruit weight	NS	NS	NS	NS
Internode distance	*	NS	NS	NS

NS - not significant at 5% level

*, **, *** - significant at the 5%, 1% and .1% probability level respectively

TABLE 5.18.

MEANS OF SOME CHARACTERS FOR A AND B PLANTS (OF *X. STRUMARIUM*) IN ENVIRONMENTS B AND E

Character	Seeds	B	E
Number of clusters	A	8.7	201.3
	B	7.8	106.6
Number of branches	A	0.1	20.1
	B	0.0	13.2
Leaf width (mm)	A	105.2	160.1
	B	112.5	131.2
Leaf length (mm)	A	105.3	141.9
	B	111.7	122.1
Number of fruit	A	15.9	675.5
	B	16.3	400.8

B plant means in Environment E (Table 5.18). The data indicate that A plants tended to be bigger than B plants for all 4 races of *X. strumarium* especially in Environment E. The significant interaction between seeds and populations for fruit length was due to the fruit of B plants being significantly larger than those of the A plants in both environments for *X. cavanillesii* only. This was partially reflected in the bigger fruit weight of the B plants in Environment E, though this difference was not significant. Perhaps these differences were due to 3 seed formation preferentially in the B plants, but this possibility has not been investigated.

5.4. DISCUSSION

The results demonstrated that the relative amounts of phenotypic plasticity in the races of *X. strumarium* and *X. spinosum* were different, and in some cases markedly so. Thus for the particular characters and environments used in the present study *X. chinense* showed greater plasticity than *X. pennsylvanicum*, which in turn had more than *X. cavanillesii*. *X. italicum* had similar levels of plasticity to *X. chinense* while generally *X. spinosum* had higher levels than *X. strumarium*. Nevertheless, all the types exhibited considerable plasticity for the range of environments used. The differences in plasticity between races were only quantitative in the sense that overall, with perhaps the exception of *X. cavanillesii*, the plasticity was a much larger component of the total phenotypic variation than the genetic variation. Moreover, the between-plant variance (σ_G^2) is most likely an overestimate of genetic quantitative variation, since microenvironmental sources of variation will be included in this variance term. The size of the microenvironmental component is undoubtedly small in most environments but the actual size is unknown and has been assumed to be equal for all populations.

For 3 of the races the predicted negative correlation between plasticity and genetic variation [Levins, 1963] was confirmed. Thus, *X. chinense* had a higher relative plasticity than *X. pennsylvanicum* and *X. cavanillesii* but a lower estimate of genetic variation. *X. italicum* did not fit this strategy so readily, since although it had a higher genetic variation than *X. chinense* it had similar levels of plasticity. In contrast the other species, *X. spinosum* appears to have adopted a different strategy with relatively higher amounts of both plasticity and genetic variation.

The Split-plot analyses showed that the source of the differences in plasticity between the races of *X. strumarium* originated almost entirely from Environments B, C, D, E and not from Environment A. In Environment A, the photoperiod was controlled with the result that all races had similar juvenile phases and life cycles of the same length. However, in the other environments the length of the effective life cycles, including the juvenile phases, were different, being determined by the different short day requirements of the races. The supposed differences in critical night length requirements between *X. italicum* and *X. chinense* [McMillan, 1975b] resulted in only small differences between the dates of initiation of flowering at the latitude of Canberra.

Both *X. pennsylvanicum* and *X. cavanillesii* which had much shorter juvenile phases compared to *X. chinense* had lower levels of plasticity as well. It seems that the genes involved in the photoperiodic control of flowering, indirectly at least, determine the level of phenotypic plasticity that can be expressed. In other words, the environment both controls the expression of flowering and concomitantly sets limits on the range of the plasticity possible by defining the length of the juvenile phase of the life cycle. Populations then occupy positions in this range as a result of other environmental factors. Therefore at any given latitude a race could be thought of as having a theoretical maximum plasticity, but this theoretical maximum would vary over latitudes. The question may well be that if the photoperiodic control of flowering was removed would the races have the same potential plasticity or not?

X. pennsylvanicum and perhaps *X. cavanillesii* appeared to have higher growth rates than *X. chinense*, from the data of Enviroment A, and this may be adaptation to compensate for the lower critical night length and subsequent shorter life cycle. The higher growth rate is most likely an adaptation of the race to its native habitat, rather than adaptation to its place of introduction, since for instance *X. pennsylvanicum* must complete its life cycle in the short summers of northern California. Since the plasticity potential of some of the races is largely overridden by photoperiod in normal field situations it seems feasible that an alternative strategy has been adopted as well - namely direct genetic control of the quantitative variation.

The resultant mixed strategies would account for the higher estimates of genetic variation for *X. pennsylvanicum* and *X. cavanillesii* compared to *X. chinense*, and it is envisaged that the genetic variation resulted from selection in native habitats. For the environments and the latitude in which the experiment was performed, *X. italicum* showed similar plasticity to *X. chinense*, whereas in environments where the change in night length from 10-11 hours takes several weeks (e.g. south eastern United States of America from where the Australian *X. italicum* apparently came from, or northern Queenland), then the plasticity could well be greater for *X. chinense*. Thus *X. italicum* may have evolved towards quantitative genetic variation in its native habitats adopting a mixed strategy on phenotypic variation in the process, like the other 2 races and in contrast to *X. chinense*.

In Chapter 4, field data showed that *X. italicum* had higher levels of quantitative variation than the other races. Thus, in the light of the experimental results of this Chapter, the overall phenotypic variation observed in the 2 *X. italicum* populations was larger than in the populations of *X. chinense* primarily because of the higher genetic

component in the former. Similarly the phenotypic variation in the Mildura population (59) of *X. pennsylvanicum* was less than in *X. italicum* populations due largely to the higher plasticity in the *X. italicum* race.

Strategies r and k are really only comparative though Pianka [1970] listed the distinguishing characteristics of the two extreme types. It was shown that *X. chinense* was characterized by a tendency for higher fruit (seed) output than *X. italicum* in the Environments A, B and C, which in many aspects covered the range of suitable environments occurring in western New South Wales. This suggests that *X. chinense* would be a more successful r-strategist in these irregular and somewhat harsh environments and that perhaps *X. italicum* has not successfully colonized these areas because it cannot build up a large enough seed bank to survive the unfavourable seasons.

Whether the large amounts of plasticity in the species have an adaptive value or not cannot be proven directly from this data. However, the differences in plasticity estimates between some populations within races at least indirectly, suggests that some adaptation in plastic responses may have occurred since introduction into Australia. Thus, the trend of lower plasticity levels for most characters of population 11 (latitude 22°) may represent an adaptation to the short life cycle of the more northern latitude. On the other hand, there were definite trends in the plasticity levels between populations of *X. spinosum* which were from only slightly different latitudes, and yet the species is supposedly photoperiodically neutral [Lona, 1946]. There was an apparent correlation between the plasticity in these populations and the size of the original plants at the population sites. Thus population 23 (Deniliquin) had overall the largest plasticity, and also the plants in the original field population were all very large, each having at least several hundred seeds; whereas plants in population

27 (Wilpena) were all very small, averaging less than 30 fruit per plant due to the very dry harsh conditions. The experimental results indicated that this population tended to have lower plasticity estimates than population 23. More likely these differences are partly a result of the lower genetic variation of population 23 compared to 27 rather then just different adaptations in plasticity.

Flowering in *X. spinosum* seems to be controlled by a ripeness to flower response or a maturity factor. It was observed that small plants tended to flower later than bigger plants, especially in the field Environments D and E, and this was also a common phenomenon field populations. Thus in field populations when there is some difference in age structure then flowering will occur over a relatively wide time span in constrast to the situation in the races of *X. strumarium*.

Characters had distinctive levels of plasticity and whether a combination of these different levels represented an optimal strategy for the species of *Xanthium* was not discernible from the present study.

The results of the subsidiary experiment showed that the A plants tended to have higher means for several characters in either both environments or Environment E only. The data indicated that the B plants had the same magnitude of variation between environments as the A plants. The B plants have lower means in both environments for many of the characters, so they could in fact have the same environmental variance. Thus A and B plants exhibit large but similar plasticities but the plasticities are over somewhat different range. This tends to confirm McHargue's [1921] conclusions that "the large seeds produce larger and more vigorous plants than the small seeds." It must be emphasized that overall the A plant means were greater than the B plant means for all 4 races of *X. strumarium*. This does not necessarily

mean that there are genetic differences between and A and B seeds since the different plasticity responses could be of a non-adaptive nature. Whether or not the B plants had similar amounts of quantitative genetic variation to the A plants could not be determined from the analyses carried out. The replicates for A and B plants were physically of different size since A replicates also had *X. spinosum* in them. This could perhaps have resulted in less competition for light etc. in the A replicates compared to the B replicates with the result of bigger plants in A replicates.

Field observations showed that there was a dichotomy in size of A and B plants at high density (plants similar in size to plants of Environment B). This dichotomy could only have partly been due to differences like those demonstrated in the above experiments and was largely a result of differential germination of the seeds and subsequent intraspecific competition.

CHAPTER 6

General Discussion and Conclusions

The two species of the genus *Xanthium* are successful colonizers of large regions of Australia. Their distributions overlap but *X. spinosum* occupies a more temperate range. The 4 races of *X. strumarium* have very different geographical ranges although *X. chinense* is sympatric with the other 3 races but in separate parts of its range. *X. spinosum* is photoperiodically day neutral with respect to flowering but *X. strumarium* is a short day plant, and the races established in Australia have different critical night length requirements for flowering.

There was remarkable absence of allozyme polymorphisms within populations of *X. spinosum* and 3 of the races of *X. strumarium*. This is in contrast to the substantial allozyme variation reported in almost all other organisms. The 7 *X. chinense* populations were monomorphic at all loci scored. As well, the populations of the races of *X. cavanillesii* and *X. pennsylvanicum* were monomorphic at all loci scored. For the loci analysed all members of the 3 populations of *X. spinosum* had only 1 genotype. Data indicating such a complete lack of genetic variation has not been reported for plants before. In *Oenothera biennis* only a few genotypes are present, but this is as a result of a special chromosomal system of permanent translocation heterozygosity [Levin, 1975b]. The high qualitative genetic uniformity over the large ranges that the *Xanthium* species have colonized has not been documented for any other plant species, even for colonizers. A situation approaching

this, is that of *A. barbata* in California, where 2 genotypes predominate, and these are found in different ecological habitats [Clegg *et al*, 1972].

The implication from the data is that allozyme variation is not necessary for successful colonization of vast areas of Australia by species of the *Xanthium* genus. *X. chinense* has no detectable qualitative variation and relatively low estimates of quantitative genetic variation and yet, despite this or perhaps partly because of it, the race has spread over most of eastern Australia. The two simplest explanations are that (1) there were very few individuals introduced for each race and no allozyme variation was present in the individuals or (2) allozyme variation was present in the introductions but there was strong selection for particular alleles at each of the loci in each of the races and species. This latter explanation seems very unlikely. *X. chinense* and *X. spinosum* spread rapidly on introduction and occupied wide geographic ranges before 1900, and if selection pressures were so intense one would have expected genetic differentiation between populations with the range of environments encountered by the species. This explanation also implies that allozyme variation is not necessary for colonization, but that the fixed alleles are strongly selected for.

The more likely explanation of the allozyme data is that the alleles are selectively neutral and have no functional role in a fitness sense. Until recently, viewpoints in favour of selective neutrality have been based on Kimura and Crow's [1964] neutral infinite allele model. It has become clear, however, that this model is inappropriate for allozyme data, and as a result there has been the development of the neutral charge class model [Ohta and Kimura, 1974]. Ohta [1975] and Latter [1975] have compared observed protein polymorphisms in *Drosophila* with the expected gene frequency distributions on the basis of the neutral charge class model. They found an excess of rare alleles in the

observed distributions but these fitted the expected distributions very well if all mutations are very slightly disadvantageous. Under the neutral charge class model mutant alleles in effect "bank up" in a population, since selection pressures against them are not high enough to eliminate them immediately. Even assuming no allozyme variation in the original introductions, the two most successful types have both been in Australia over a hundred years, which is time enough for mutants at the allozyme loci to accumulate in the proposed manner.

The lack of allozyme variation in *Xanthium* can be explained by the neutral charge class model if either (1) selection against new mutants is higher in the genus *Xanthium*, but random drift removes the rare alleles. There are some indications that the first alternative is somewhat unlikely since evolution in polyploids is considered to be often slower than in normal diploids and that concomitantly mutation and selection pressures are not as great [Stebbins, 1970]. *Xanthium* is tetraploid only in relation to the family chromosome number of n = 9, not to the tribe Ambrosiinae, which has a basic number n = 18 [Payne *et al*, 1964]. As well *Xanthium* has probably 2 copies of each gene so it might be argued that selection against a mutant at one of these would, if anything, be less.

Xanthium species occur in very fluctuating and irregular ecological habitats and are completely dependent on favourable seasons for continuity of populations. It has been commonly observed that there are enormous fluctuations in the size of populations, not only in terms of area but more importantly the number of plants. Under such conditions "bottleneck" could arise and random drift would effectively remove the rare mutant alleles with a resultant genetic uniformity in qualitative variation in the *Xanthium* species.

There has been no previous studies reporting the level of allozyme variation in plant species which occupy fluctuating and highly unstable habitats. The few colonizing species that have been examined for allozyme variation, have become established in their new environments, and form fairly stable populations e.g. *Avena* species in California [Jain, 1969; Clegg and Allard, 1972]. In marked contrast the *Xanthium* species in most of the ecological situations effectively recolonize areas only in every favourable season. One would predict then that if the majority of allozymes are selectively neutral and are maintained by mutation-selection balance without recourse to any form of balancing selection, species living in fluctuating environments with regular "bottlenecks" in population size would have low genetic allozyme variation. This effect of random drift on allozyme variation within populations would be expected to be maximized in small introductions of inbreeding colonizers.

Another factor tending to reduce genetic differentiation within both species is the gene flow between populations. The highly efficient dispersal mechanisms of *Xanthium* fruit, namely water and stock, can cause considerable gene flow over much of the range of the species, and this would be a lot higher than has normally been envisaged for plant species [Bradshaw, 1972]. Several explanations of the maintenance of the higher allozyme variation in the *X. italicum* populations are possible. Comparison of the inbreeding coefficients and the fixation indices indicated that the frequency of heterozygotes was higher than expected and this suggests heterozygote advantage. However, the data could be explained in terms of selective neutrality, especially since the *X. italicum* complex includes a very broad group of plants [Love and Dansereau, 1959; McMillan, 1975b], and hence the allozyme variation may well have been present on introduction. The polymorphism at the

EST-4 locus may be still present because at least 2 alleles were common in the introduction and these have survived "bottlenecks". Estimates of t, the rate of outcrossing, indicated that *X. strumarium* is a highly inbreeding species.

All races of *X. strumarium*, as well as *X. spinosum*, exhibit extensive phenotypic variation in natural populations. *X. chinense* has lower levels of quantitative genetic variation than *X. spinosum* and the other 3 races of *X. strumarium*. This is in marked contrast to the allozyme variation, since for the latter only *X. italicum* had polymorphic loci. If the quantitative genetic variation was neutral as allozyme variation appears to be, then the effect of drastic changes in population size would be to make the levels of quantitative genetic variation similar for all races. The fact that it is lower for *X. chinense* compared to the other races and that *X. spinosum* is higher than *X. strumarium* suggests that these levels were selected for, or at least maintained in their Australian environments.

The races of *X. strumarium* have different short day requirements for flowering most likely as a result of adaptation to their native habitats. *X. strumarium* is tropical or subtropical in origin with a preference for dry habitats. There is intense selection pressure to complete the life cycle within the warm summer season and produce seed and since (1) the species is apparently intrinsically incapable of adapting to cold conditions then (2) the length of the life cycle is adjusted by changing the time of initiation of flowering. In reality, this adaptation in critical night length, although ensuring some seed production, limits the expression of the potential plasticity in many cases, such that these races switch partly to another strategy, namely direct genetic control of the quantitative variation. The higher quantitative genetic variation of *X. cavanillesii*, *X. pennsylvanicum* and

X. italicum compared to *X. chinense* probably originated as a consequence of the adaptation in critical night length to ensure seed production, before the onset of winter and continuation of the species to the next generation. It seems likely therefore that these races introduced to Australia were adapted to more restricted environments than *X. chinense*. McMillan [1975b] has shown that over the range of native habitats most of the races each have a range of critical night lengths, which are determined by the latitude of the population site. If the interpretations of the data are correct, then within *X. chinense* for instance, the native populations with lower critical night length requirements (e.g. 9 hours) would be expected to have higher levels of quantitative genetic variation than the introduced Australian strain. *X. spinosum* also is of a subtropical origin, but has a more temperate distribution. This is probably a reflection of the alternative strategy employed by this species, since it can become established in wetter, colder environments. Observations suggest it has some degree of frost resistance and in fact, *X. spinosum* does not require photoperiodic control of flowering to ensure seed production.

The highly unstable nature of the environments encountered by *Xanthium* species leads to considerable wastage of seed by various processes. It is thus an optimal strategy to maximize seed production wherever possible. This *X. chinense* and *X. spinosum* can do successfully over wide geographic ranges. The studies on seed population dynamics revealed that a large part of the variation in germination response was environmentally induced. *X. chinense* had the largest germination response over the whole range of environments compared to *X. italicum* and *X. pennsylvanicum*. *X. pennsylvanicum* had the most restricted germination response in that it required a prechilling phase to achieve substantial germination in any of the environments and this appears to be due to genetic control of the germination of the A seeds.

There was higher total variation in germination response between populations of *X. spinosum* than those of *X. chinense*, and this probably indicates higher adaptive response in plasticity for these processes in *X. spinosum*. The manner of control of the between plant variation is not clear.

Seeds of *Xanthium* can show 2 germination flushes within a season - one immediately after favourable environmental conditions and the other later in summer brought on by the drying of seed and subsequent favourable moisture conditions. In the first germination flush, the germination of non-dormant seeds is particularly high in optimal environmental conditions compared to other weedy species. On the other hand, only a comparatively small fraction of the dormant seeds germinate in the first season and these are primarily in the second flush of germination. All *Xanthium* species exhibit seed dormancy. This seed dormancy clearly provides a buffering system enabling part of a population to escape environmental disasters such that if the season is subsequently adverse and growth and seed production low the conditions inducing this will also prevent further germination through the season. In effect, there is an inbuilt timing of germination to maximize reproduction in a very fluctuating environment.

The seed carryover to the next season is primarily of dormant (B) seeds, and its size varies between environments, but generally it increases as environmental temperature decreases, when other factors such as moisture are non-limiting. In fact, in the drier western parts of the distribution of *X. chinense*, accumulated seed loss over bad seasons must be considerable and probably *X. pennsylvanicum* and *X. cavanillesii* could not maintain a sufficiently high seed bank in the soil to successfully colonize these areas over a period of time. The length of viability of *Xanthium* seed in soil in these areas appears to

be less than 5 years [Mann, 1965] but the length of viability of seeds on the soil surface is not known, but would be considerably longer. In summary, *X. chinense* has the highest germination response overall in the environments studied while *X. pennsylvanicum* had the least.

X. chinense has the lowest quantitative genetic variation and no allozyme variation has been detected within this race, yet it is by far the most successful colonizer of the races of *X. strumarium* introduced into Australia. *X. chinense* had greater amounts of phenotypic plasticity relative to *X. cavanillesii* and *X. pennsylvanicum*. It was also argued that because of the widening gap in the length of the life cycles of *X. italicum* and *X. chinense* with decreasing latitude, *X. chinense* would have greater plasticity than *X. italicum* over a large proportion of its range. As a result of its large short day requirement, *X. chinense* had ample plasticity to cope successfully with fluctuating environments without recourse to extensive genetic variation.

The two species have employed somewhat different strategies of colonization. *X. spinosum* has high levels of both plasticity and genetic variation, whereas *X. chinense* has a comparatively high plasticity level but a markedly low level of genetic variation. As well, *X. spinosum* appears to have shown more adaptation in plastic response of germination compared to the races of *X. strumarium*. For the races of *X. strumarium* the broader the niche of the race the lower is the genetic quantitative variation and concomitantly the higher the plasticity. At least when comparing the races of *X. strumarium* by means of quantitative characters the genetic variation per race decreases as the range of environmental variation increases. The data tend to confirm Lewontin's [1965] predictions that the lower the genetic variability of a species the more successful it will be in colonizing irregular and unstable environments. Such species can have an alternative strategy of high

plasticity and this would be maximized in inbreeding annuals since genetic flexibility for adaptation to fluctuating environments demands recombination to generate adaptive combinations different from those previously selected. Highly inbreeding species have low rates of outcrossing and hence little recombination and thus they would adopt a strategy of maximum plasticity. In addition, the data for *X. strumarium* tended to confirm Levins' [1963] predictions that there was an in verse relationship between plasticity and genetic variation.

In this study it was found:

(1) Allozyme variation was not necessary for successful colonization of Australia by *X. spinosum* and at least 3 of the races of *X. strumarium*.

(2) Except for *X. italicum* the only allozyme variation was between races and species.

(3) The 2 most successful colonizers namely *X. spinosum* and *X. chinense* have different genetic strategies of colonization.

(4) The different strategies employed by the races of *X. strumarium* were most likely evolved in their native habitats in response to strong selection pressures.

(5) The races of *X. strumarium* were primarily preadapted to the ranges they successfully colonized in Australia.

(6) All species exhibited considerable phenotypic plasticity but *X. chinense* was the most successful of the races because it not only has high plasticity but low genetic variation as well.

(7) There was little evidence of genetic adaptation by the races of *X. strumarium* since introduction, yet there was some indication of adaptive changes in the levels of plasticity for phenotypic characters.

(8) Both species exhibit considerable plasticity in germination and dispersal mechanisms. All races and species show a dormancy of the upper seeds of fruit and this appears to be somatically controlled fixed

variation. All three processes contribute considerably to the success of these species as colonizers of Australian environments.

APPENDIX A

FORMULAE FOR BUFFERS USED

BUFFER I: Tris-citrate buffer (pH 8.2)

Tris (hydroxymethyl) aminomethane	=	7.871 g
Citric acid	=	1.921 g
Water	=	1 litre

BUFFER II: Lithium-borate buffer (pH 8.5)

Lithium hydroxide	=	2.098 g
Boric acid	=	11.800 g
Water	=	1 litre

BUFFER III: Histidine buffer (pH 8.0)

Histidine hydrochloride	=	1.05 g
Water	=	1 litre

pH adjusted to 8.0 with 10N NaOH

BUFFER IV: Tris-versene-borate buffer (pH 8.0)

Tris (hydroxymethyl) aminomethane	=	60.6 g
Boric acid	=	40.0 g
Na_2EDTA	=	6.0 g
Water	=	1 litre

BUFFER V: Tris-citrate buffer (pH 8.7)

Tris (hydroxymethyl) aminomethane	=	4.598 g
Citric acid	=	.525 g
Water	=	1 litre

BUFFER VI: Sodium citrate buffer (pH 8.0)

Sodium citrate	= 118.9 g
Water	= 1 litre

pH adjusted to 8.0 with 0.41M citric acid

BUFFER VII: Tris-boric acid tank buffer (pH 8.0)

Tris (hydroxymethyl) aminomethane	= 6.06 g
Boric acid	= 6.00 g
$Na_2EDTA.2H_2O$	= .60 g
Water	= 1 litre

BUFFER VIII: Borax-borate buffer (pH 8.8)

Borax	= 63.0 g
Boric acid	= 28.88 g
Water	= 1 litre

BUFFER IX: Phosphate buffer A (pH 8.8)

Disodium hydrogen phosphate	= 71.632 g
Water	= 1 litre

BUFFER X: Phosphate buffer B (pH 4.6)

Sodium dihydrogen phosphate	= 31.202 g
Water	= 1 litre

BUFFER XI: Tris maleic buffer (pH 3.7)

Tris (hydroxymethyl) aminomethane	= 24.22 g
Maleic anhydride	= 19.612 g
Water	= 1 litre

BUFFER XII: Sodium-alkali buffer (pH 12.2)

Sodium hydroxide	=	6.00 g
Water	=	1 litre

BUFFER XIII: Acetate buffer (pH 4.9)

Sodium acetate	=	11.485 g
Acetic acid (glacial)	=	3.6 ml
Water	=	1 litre

APPENDIX B

DETAILS OF SPECIFIC ELECTROPHORETIC SYSTEMS

System	Type	Gel buffer	Tank buffer
1	Starch	Buffer I, II	Buffer II
2	Starch	Buffer III	Buffer VI
3	Starch	Buffer IV	Buffer VII
4	Acrylamide	Buffer V	Buffer VIII

APPENDIX C

ENZYME STAINING SOLUTIONS

1. ACID PHOSPHATASES (AcPh):

Acetate buffer	= 100	ml
Fast blue RR salt	= 100	mg
Polyvinyl pyrrolidone	= 200	mg
Sodium chloride	= 1	g
10% Magnesium chloride solution	= 10	drops
α-naphthyl acid phosphate	= 100	mg

2. ALCOHOL DEHYDROGENASES (ADH):

Phosphate buffer A	= 30	ml
Phosphate buffer B	= 20	ml
Water	= 50	ml
NAD	= 10	mg
NBT	= 15	mg
PMS	= 2	mg
Ethanol	= 5	ml

3. ESTERASES (EST)

Phosphate buffer A	= 30	ml
Phosphate buffer B	= 20	ml
Water	= 50	ml
Fast blue RR salt	= 100	mg
α-naphthyl acetate (1% solution in 70% acetone)	= 1.5	ml
β-naphthyl acetate (1% solution in 70% acetone)	= 1.5	ml

4. GLUTAMATE DEHYDROGENASE (GDH)

Phosphate buffer A	= 30	ml
Phosphate buffer B	= 20	ml
Water	= 50	ml
NAD	= 10	mg
NBT	= 15	mg
PMS	= 2	mg
1M Sodium glutamate, pH 7.0	= 10	ml

5. GLUTAMATE-OXALOACETATE TRANSAMINASES (GOT)

0.2M Phosphate buffer, pH 7.5	= 5	ml
Polyvinyl pyrrolidone	= 250	mg
Pyridoxal-5-phosphate (0.05%)	= 0.2	ml
L-aspartic acid solution (2.66% adjusted to pH 7.5 with KOH)	= 1.7	ml
α-Keto-glutamic acid (1.50% adjusted to pH 7.5 with KOH)	= .7	ml

To this mixture add the following solution just before pouring it over the gel:

Fast violet B salt	= 50 mg in 2 ml water

6. GLUCOSE-6-PHOSPHATE DEHYDROGENASE (G6PDH)

.2M Tris-HCl, pH 7.1	= 100	ml
NADD	= 15	mg
NBT	= 15	mg
PMS	= 2	mg
Disodium glucose 6 phosphate.H_2O	= 60	mg

7. LEUCINE AMINOPEPTIDASES (LAP)

Tris maleic anhydride buffer	=	50	ml
Sodium alkali buffer	=	10	ml
Water	=	40	ml
Black K salt	=	40	mg
L-leucyl-β-naphthyl-hydrochloride (dissolved in 2 ml methanol)	=	50	mg

8. MALATE DEHYDROGENASES (MDH)

.2M Tris-HCl, pH 8.0	=	90	ml
NAD	=	10	mg
NBT	=	15	mg
PMS	=	2	mg
1M sodium malate, pH 8.0	=	10	ml

APPENDIX D: Results of the Split-plot analysis of variance of data for 11 characters for the 10 populations

(a) Basal diameter

Source	DF	S.S.	M.S.	F
Env.	4	3.1496	.7874	76.67$^{\phi}$
Reps. Env. Error	5	.0514	.0103	
Popn.	9	.5965	.0063	21.31$^{\phi}$
Env. x Popn.	30	.2856	.0095	3.06$^{\phi}$
Error	39	.1211	.0031	
Total	87	4.2042		

(b) Plant height

Source	DF	S.S.	M.S.	F
Env.	4	1.2877	.3219	60.97$^{\phi}$
Reps. Env. Error	5	.0264	.0053	
Popn.	9	.1817	.0202	6.71
Env. x Popn.	30	.3461	.0115	3.83$^{\phi}$
Error	39	.1174	.0030	
Total	87	1.9593		

$^{\phi}$ - significant at the .1% probability level

(c) Clusters

Source	DF	S.S.	M.S.	F
Env.	4	20.7943	5.1986	579.55$^{\phi}$
Reps. Env. Error	5	.0449	.0090	
Popn.	9	1.4420	.1602	9.17$^{\phi}$
Env. x Popn.	30	1.2513	.0417	2.39$^{\phi}$
Error	39	.6814	.0175	
	87	24.2139		

(d) Number of branches

Source	DF	S.S.	M.S.	F
Env.	4	20.2118	5.0530	842.16$^{\phi}$
Reps. Env. Error	5	.0300	.0060	
Popn.	9	1.2068	.1341	15.89$^{\phi}$
Env. x Popn.	30	.9894	.0330	3.91$^{\phi}$
Error	39	.6054	.0084	
	87	23.0434		

$^{\phi}$ - significant at the .1% probability level

(e) Number of minor leaves

Source	DF	S.S.	M.S.	F
Env.	4	10.1722	2.5431	105.61$^{\phi}$
Reps. Env. Error	5	.1204	.0241	
Popn.	9	3.5767	.3426	39.74$^{\phi}$
Env. x Popn.	30	1.8779	.0626	6.26$^{\phi}$
Error	39	.3900	.0100	
	87	16.1372		

(f) Number of major leaves

Source	DF	S.S.	M.S.	F
Env.	4	11.7389	2.9347	118.43$^{\phi}$
Reps. Env. Error	5	.1239	.0248	
Popn.	9	1.9039	.2115	19.23$^{\phi}$
Env. x Popn.	30	.7508	.0250	2.28$^{\phi}$
Error	39	.4289	.0110	
	87	14.9464		

$^{\phi}$ - significant at the .1% probability level

(g) Number of fruit

Source	DF	S.S.	M.S.	F
Env.	4	37.7218	9.4305	392.12$^{\phi}$
Reps. Env. Error	5	.1202	.0241	
Popn.	9	3.0838	.3426	11.81$^{\phi}$
Env. x Popn.	30	1.7834	.0595	2.05*
Error	39	1.1313	.0290	
	87	43.8405		

(h) Plant weight

Source	DF	S.S.	M.S.	F
Env.	4	40.2197	10.0549	538.27$^{\phi}$
Reps. Env. Error	5	.0934	.0187	
Popn.	9	4.0397	.4489	14.833$^{\phi}$
Env. x Popn.	30	2.2360	.0745	2.46^{+}
Error	39	1.8029	.0303	
Total	87	48.3917		

*, +, ϕ - significant at the 5%, 1% and .1% probability level respectively

(i) Days to flowering

Source	DF	S.S.	M.S.	F
Env.	4	9836.7095	2459.1774	2049.31$^{\phi}$
Reps. Env. Error	5	5.8000	1.2000	
Popn.	9	21837.4286	2426.3809	1452.92$^{\phi}$
Env. x Popn.	30	6533.7571	217.7919	130.41$^{\phi}$
Error	39	65.2000	1.6700	
Total	87	38278.8952		

(j) Time of anthesis

Source	DF	S.S.	M.S.	F
Env.	4	16479.0905	4119.7226	1525.84$^{\phi}$
Reps. Env. Error	5	14.7000	2.7000	
Popn.	9	25979.6667	2887.6296	96.39$^{\phi}$
Env. x Popn.	30	8097.1762	269.9059	90.12$^{\phi}$
Error	39	116.8000	2.9949	
Total	87	50687.4334		

$^{\phi}$ - significant at the .1% probability level

(k) Internode distance

Source	DF	S.S.	M.S.	F
Env.	4	.9328	.2332	63.37$^{\phi}$
Reps. Env. Error	5	.0184	.0037	
Popn.	9	1.0591	.1177	46.33$^{\phi}$
Env. x Popn.	30	.2879	.0096	3.78$^{\phi}$
Error	39	.0990	.0025	
Total	87	2.3972		

$^{\phi}$ - significant at the .1% probability level

APPENDIX E: Results of the Split-plot analysis of variance of 4 characters for the 7 populations of *X. Strumarium*

(a) Leaf width

Source	DF	S.S.	M.S.	F
Env.	4	26069.37	6517.34	46.83$^{\phi}$
Reps. Env. Error	5	695.79	139.16	
Popn.	6	17975.54	2995.92	31.63$^{\phi}$
Env. x Popn.	24	11115.03	463.13	4.89$^{\phi}$
Error	30	2841.71	94.72	
Total	69	58697.44		

(b) Leaf length

Source	DF	S.S.	M.S.	F
Env.	4	11456.80	2864.20	49.37$^{\phi}$
Reps. Env. Error	5	290.07	58.01	
Popn.	6	7181.69	1196.95	14.97$^{\phi}$
Env. x Popn.	24	6463.60	269.32	3.36*
Error	30	2398.43	79.95	
Total	69	27790.59		

*,ϕ - significant at the 5% and .1% probability level respectively

(c) Fruit length

Source	DF	S.S.	M.S.	F
Env.	4	20.9120	5.2280	5.09$^{\phi}$
Reps. Env. Error	5	5.1286	1.0257	
Popn.	6	152.4634	24.4106	106.74$^{\phi}$
Env. x Popn.	24	80.5980	3.3582	14.11$^{\phi}$
Error	30	7.1414	0.2380	
Total	69	266.2434		

(d) Fruit weight

Source	DF	S.S.	M.S.	F
Env.	4	0.1557	0.0389	12.87$^{\phi}$
Reps. Env. Error	5	0.0151	0.0030	
Popn.	6	2.1805	0.3634	190.59$^{\phi}$
Env. x Popn.	24	0.4517	0.0188	9.86$^{\phi}$
Error	30	0.0572	0.0019	
Total	69	2.8603		

$^{\phi}$ - significant at the .1% probability level

APPENDIX F: Results of Split-plot analysis of variance of 4 characters for 3 populations of *X. spinosum*

(a) Leaf width

Source	DF	S.S.	M.S.	F
Env.	2	140.11	70.06	33.18+
Reps. Env. Error	3	6.33	2.11	
Popn.	2	33.79	16.89	13.22+
Env. x Popn.	4	20.56	5.14	4.02
Error	6	7.67	1.28	
Total	17	208.46		

(b) Leaf length

Source	DF	S.S.	M.S.	F
Env.	2	2094.33	1047.17	49.87+
Reps. Env. Error	3	63.00	21.00	
Popn.	2	66.33	33.17	5.24*
Env. x Popn.	4	78.33	19.58	3.09
Error	6	38.00	6.33	
Total	17	2339.99		

*, + - significant at the 5% and 1% probability level respectively

(c) Fruit length

Source	DF	S.S.	M.S.	F
Env.	2	3.88	1.94	8.84
Reps. Env. Error	3	0.66	0.22	
Popn.	2	0.57	0.29	1.99
Env. x Popn.	4	0.40	0.10	0.70
Error	6	0.86	0.14	
Total	17	6.37		

(d) Fruit weight

Source	DF	S.S.	M.S.	F
Env.	2	0.1118	0.0559	20.84*
Reps. Env. Error	3	0.0080	0.0027	
Popn.	2	0.0074	0.0037	22.27†
Env. x Popn.	4	0.0038	0.0009	5.69*
Error	6	0.0010	0.0002	
Total	17	0.1320		

*, † - significant at the 5% and 1% probability level respectively

APPENDIX G: Results of Split-plot analysis of variance of characters obtained from subsidiary experiment (A x B plants)

(a) Basal diameter

Source	DF	S.S.	M.S.	F
Env.	1	3.0560	3.0560	1210.547$^{\phi}$
Seeds	1	0.0113	0.0113	4.487
Env. x Seeds	1	0.0061	0.0061	2.410
Error 1	4	0.0101	0.0025	
Popn.	6	0.1597	0.0266	5.277$^{\phi}$
Env. x Popn.	6	0.0922	0.0154	3.047*
Seeds x Popn.	6	0.0479	0.0080	1.583
Env. x Seeds x Popn.	6	0.0408	0.0068	1.350
Error 2	24	0.1210	0.0050	
Total	55	3.5451		

$^{\phi}$ - significant at the .1% probability level

(b) Plant height

Source	DF	S.S.	M.S.	F
Env.	1	0.6704	0.6704	212.051$^{\phi}$
Seeds	1	0.0491	0.0491	15.536*
Env. x Seeds	1	0.0082	0.0082	2.607
Error 1	4	0.0126	0.0032	
Popn.	6	0.0672	0.0112	3.114*
Env. x Popn.	6	0.0288	0.0048	1.335
Seeds x Popn.	6	0.0238	0.0040	1.102
Env. x Seeds x Popn.	6	0.0265	0.0044	1.227
Error 2	24	0.0863	0.0036	
Total	55	0.9729		

(c) Number of clusters

Source	DF	S.S.	M.S.	F
Env.	1	20.2838	20.2838	947.197$^{\phi}$
Seeds	1	0.3426	0.3426	15.999*
Env. x Seeds	1	0.1931	0.1931	9.016
Error 1	4	0.0857	0.0214	
Popn.	6	0.7720	0.1287	4.921^{+}
Env. x Popn.	6	0.7070	0.1178	4.507^{+}
Seeds x Popn.	6	0.2465	0.0411	1.571
Env. x Seeds x Popn.	6	0.1773	0.0296	1.130
Error 2	24	0.6275	0.0261	
Total	55	23.4355		

*, $^{+}$, $^{\phi}$ - significant at the 5%, 1% and .1% probability level respectively

(d) Number of branches

Source	DF	S.S.	M.S.	F
Env.	1	20.8101	20.8101	9349.320$^{\phi}$
Seeds	1	0.1286	0.1286	57.764^{+}
Env. x Seeds	1	0.0795	0.0795	35.733^{+}
Error 1	4	0.0089	0.0022	
Popn.	6	0.3475	0.0579	3.608*
Env. x Popn.	6	0.1954	0.0326	2.029
Seeds x Popn.	6	0.2165	0.0361	2.248
Env. x Seeds x Popn.	6	0.1887	0.0315	1.959
Error 2	24	0.3853	0.0161	
Total	55	22.3605		

(e) Leaf width

Source	DF	S.S.	M.S.	F
Env.	1	18944.64	18944.64	98.268$^{\phi}$
Seeds	1	1628.64	1628.64	8.448*
Env. x Seeds	1	4572.07	4572.07	23.716^{+}
Error 1	4	771.14	192.79	
Popn.	6	21296.50	3549.42	44.348
Env. x Popn.	6	1617.86	269.64	3.369*
Seeds x Popn.	6	1801.36	300.23	3.751^{+}
Env. x Seeds x Popn.	6	2015.43	335.90	4.197^{+}
Error 2	24	1920.86	80.04	
Total	55	54568.50		

*, $^{+}$, $^{\phi}$ - significant at the 5%, 1% and .1% probability level respectively

(f) Leaf length

Source	DF	S.S.	M.S.	F
Env.	1	7708.02	7708.02	122.452$^{\phi}$
Seeds	1	624.45	624.45	9.920*
Env. x Seeds	1	2405.16	2405.16	38.210^{+}
Error 1	4	251.79	62.95	
Popn.	6	11114.61	1852.43	21.295$^{\phi}$
Env. x Popn.	6	787.61	131.27	1.509
Seeds x Popn.	6	1057.68	176.28	2.026
Env. x Seeds x Popn.	6	832.96	138.83	1.596
Error 2	24	2087.71	86.99	
Total	55	26869.99		

(g) Number of minor leaves

Source	DF	S.S.	M.S.	F
Env.	1	11.3956	11.3956	809.567$^{\phi}$
Seeds	1	0.2406	0.2406	17.094*
Env. x Seeds	1	0.0128	0.0128	0.908
Error 1	4	0.0563	0.0141	
Popn.	6	0.8452	0.1409	7.395$^{\phi}$
Env. x Popn.	6	1.6249	0.2708	14.218$^{\phi}$
Seeds x Popn.	6	0.1542	0.0257	1.349
Env. x Seeds x Popn.	6	0.1193	0.0199	1.044
Error 2	24	0.4572	0.0191	
Total	55	14.9061		

*, $^{+}$, $^{\phi}$ - significant at the 5%, 1% and .1% probability level respectively

(g) Number of minor leaves

Source	DF	S.S.	M.S.	F
Env.	1	11.3956	11.3956	809.567$^{\phi}$
Seeds	1	0.2406	0.2406	17.094*
Env. x Seeds	1	0.0128	0.0128	0.908
Error 1	4	0.0563	0.0141	
Popn.	6	0.8452	0.1409	7.395$^{\phi}$
Env. x Popn.	6	1.6249	0.2708	14.218$^{\phi}$
Seeds x Popn.	6	0.1542	0.0257	1.349
Env. x Seeds x Popn.	6	0.1193	0.0199	1.044
Error 2	24	0.4572	0.0191	
Total	55	14.9061		

(h) Number of major leaves

Source	DF	S.S.	M.S.	F
Env.	1	11.7137	11.7137	2917.500$^{\phi}$
Seeds	1	0.0402	0.0402	10.023*
Env. x Seeds	1	0.0248	0.0248	6.172
Error 1	4	0.0161	0.0040	
Popn.	6	0.9047	0.1508	11.223$^{\phi}$
Env. x Popn.	6	0.1165	0.0194	1.445
Seeds x Popn.	6	0.1753	0.0292	2.174
Env. x Seeds x Popn.	6	0.1319	0.0220	1.636
Error 2	24	0.3224	0.0134	
Total	55	13.4456		

*, $^{\phi}$ - significant at the 5% and .1% probability level respectively

(i) Number of fruit

Source	DF	S.S.	M.S.	F
Env.	1	30.9647	30.9647	1803.937$^{\phi}$
Seeds	1	0.1745	0.1745	10.164*
Env. x Seeds	1	0.2025	0.2025	11.800*
Error 1	4	0.0687	0.0172	
Popn.	6	1.9969	0.3328	7.886$^{\phi}$
Env. x Popn.	6	0.3823	0.0637	1.510
Seeds x Popn.	6	0.4074	0.0679	1.609
Env. x Seeds x Popn.	6	0.1979	0.0330	0.782
Error 2	24	1.0129	0.0422	
Total	55	35.4078		

(j) Plant weight

Source	DF	S.S.	M.S.	F
Env.	1	31.9281	31.9281	1853.139$^{\phi}$
Seeds	1	0.0550	0.0550	3.191
Env. x Seeds	1	0.0623	0.0623	3.617
Error 1	4	0.0689	0.0172	
Popn.	6	1.1572	0.1929	3.791^{+}
Env. x Popn.	6	0.3875	0.0646	1.270
Seeds x Popn.	6	0.3381	0.0563	1.108
Env. x Seeds x Popn.	6	0.2965	0.0494	0.971
Error 2	24	1.2209	0.0509	
Total	55	35.5145		

*, $^{+}$, $^{\phi}$ - significant at the 5%, 1% and .1% probability level respectively

(k) Days to flowering

Source	DF	S.S.	M.S.	F
Env.	1	658.286	658.286	449.561$^{\phi}$
Seeds	1	0.643	0.643	0.439
Env. x Seeds	1	5.786	5.786	3.951
Error 1	4	5.857	1.464	
Popn.	6	21915.250	3652.542	2425.403$^{\phi}$
Env. x Popn.	6	252.464	42.077	27.941$^{\phi}$
Seeds x Popn.	6	9.607	1.601	1.063
Env. x Seeds x Popn.	6	13.964	2.327	1.545
Error 2	24	36.143	1.506	
Total	55	22898.000		

(l) Fruit length

Source	DF	S.S.	M.S.	F
Env.	1	11.7945	11.7945	13.035*
Seeds	1	0.7545	0.7545	0.834
Env. x Seeds	1	2.3616	2.3616	2.610
Error 1	4	3.6193	0.9048	
Popn.	6	191.2861	31.8810	114.274$^{\phi}$
Env. x Popn.	6	47.0318	7.8386	28.097$^{\phi}$
Seeds x Popn.	6	4.6768	0.7795	2.794*
Env. x Seeds x Popn.	6	3.7896	0.6316	2.264
Error 2	24	6.6957	0.2790	
Total	55	272.0099		

*, $^{\phi}$ - significant at the 5% and .1% probability level respectively

(m) Fruit weight

Source	DF	S.S.	M.S.	F
Env.	1	0.0237	0.0237	6.536
Seeds	1	0.0097	0.0097	2.669
Env. x Seeds	1	0.0162	0.0162	4.456
Error 1	4	0.0145	0.0036	
Popn.	6	2.0833	0.3472	262.041$^{\phi}$
Env. x Popn.	6	0.2248	0.0375	28.275$^{\phi}$
Seeds x Popn.	6	0.0123	0.0020	1.544
Env. x Seeds x Popn.	6	0.0140	0.0023	1.763
Error 2	24	0.0318	0.0013	
Total	55	2.4303		

(n) Time to anthesis

Source	DF	S.S.	M.S.	F
Env.	1	5.786	5.786	2.160
Seeds	1	0.643	0.643	0.240
Env. x Seeds	1	0.286	0.286	0.107
Error 1	4	10.714	2.679	
Popn.	6	26494.500	4415.750	2289.648$^{\phi}$
Env. x Popn.	6	843.714	140.619	72.914$^{\phi}$
Seeds x Popn.	6	10.857	1.810	0.938
Env. x Seeds x Popn.	6	19.214	3.202	1.660
Error 2	24	46.286	1.929	
Total	55	27432.000		

$^{\phi}$ - significant at the .1% probability level

(o) Internode distance

Source	DF	S.S.	M.S.	F
Env.	1	0.1992	0.1992	38.352^{+}
Seeds	1	0.0669	0.0669	12.880*
Env. x Seeds	1	0.0038	0.0038	0.724
Error 1	4	0.0208	0.0052	
Popn.	6	0.0388	0.0065	2.825*
Env. x Popn.	6	0.0669	0.0111	4.870^{+}
Seeds x Popn.	6	0.0096	0.0016	0.701
Env. x Seeds x Popn.	6	0.0141	0.0023	1.024
Error 2	24	0.0549	0.0023	
Total	55	0.4750		

*, $^{+}$ - significant at the 5% and 1% probability level respectively

APPENDIX H: The means of the 15 characters for the 10 populations in the 5 environments

		Xanthium avanillesii	*Xanthium italicum*	*Xanthium pennsylvanicum*		*Xanthium chinense*			*Xanthium spinosum*		
Population numbers		44	46	25	26	11	22	42	23	27	43
Basal diameter (mm)	A	7.1	5.7	7.5	7.9	6.1	7.7	7.0	-	-	-
	B	6.7	6.1	6.1	5.9	7.5	7.0	7.2	4.5	4.3	4.4
	C	10.5	12.0	11.1	11.7	14.1	13.8	13.7	-	-	-
	D	10.3	16.1	12.9	10.4	15.1	13.6	15.7	8.9	7.9	6.2
	E	15.8	23.6	13.1	22.9	22.8	27.4	24.5	17.3	13.2	14.1
Plant height (cm)	A	47.9	49.5	58.2	63.8	33.2	39.6	42.0	-	-	-
	B	46.4	56.5	48.3	49.9	59.6	48.5	56.9	36.2	33.0	32.5
	C	87.9	112.4	78.3	73.3	103.8	102.8	102.0	-	-	-
	D	57.4	78.6	62.4	50.4	55.0	55.7	59.1	54.4	53.0	42.5
	E	77.3	99.7	61.6	95.4	79.4	90.8	84.2	87.0	79.0	80.1
Number of clusters	A	13.1	16.4	18.4	20.3	15.6	20.7	21.4	-	-	-
	B	6.5	8.4	11.9	13.4	9.7	9.0	9.8	9.5	9.4	9.4
	C	14.3	37.5	37.8	42.8	51.0	49.5	44.8	-	-	-
	D	39.6	175.4	63.1	59.8	86.1	95.3	109.9	81.4	57.8	49.5
	E	90.6	469.4	91.1	285.4	215.5	336.9	288.9	274.2	182.1	199.9
Number of branches	A	0.8	1.6	1.2	1.3	0.8	2.1	2.0	-	-	-
	B	0.0	0.4	0.0	0.0	0.0	0.0	0.1	0.2	0.3	0.3
	C	0.8	6.5	5.3	7.4	7.4	6.4	6.3	-	-	-
	D	4.0	13.6	6.1	5.6	6.9	8.3	8.6	10.6	8.0	5.2
	E	12.8	37.6	9.1	27.4	21.1	29.5	26.2	43.0	27.6	33.6

		Xanthium cavanillesii	*Xanthium italicum*	*Xanthium pennsylvanicum*		*Xanthium chinense*			*Xanthium spinosum*		
Population numbers		44	46	25	26	11	22	42	23	27	43
Leaf width (mm)	A	106.5	82.0	106.4	111.0	104.5	137.9	123.3	-	-	-
	B	88.4	101.0	89.1	93.0	124.8	114.2	125.4	23.7	26.7	23.6
	C	117.5	118.8	128.5	121.0	142.5	136.5	144.2	-	-	-
	D	102.4	136.5	118.9	107.4	139.0	141.0	144.6	18.9	20.3	14.7
	E	135.6	159.4	106.2	130.5	187.0	201.8	200.0	22.0	21.3	20.8
Leaf length (mm)	A	110.8	96.2	118.5	123.6	104.9	130.6	127.1	-	-	-
	B	91.8	103.4	88.1	92.2	121.3	111.9	124.3	71.0	72.8	70.8
	C	121.3	114.8	127.1	120.5	130.7	124.3	131.3	-	-	-
	D	98.4	115.6	99.0	102.1	117.9	119.5	123.1	53.3	47.5	42.1
	E	128.1	143.6	104.5	124.7	151.9	171.1	168.7	69.8	68.6	67.3
Number of minor leaves	A	38.6	39.2	43.8	49.7	42.3	50.5	42.9	-	-	-
	B	21.2	20.4	24.6	28.4	20.7	20.2	18.8	50.5	43.9	52.0
	C	32.8	88.7	61.0	67.4	77.8	81.1	77.7	-	-	-
	D	39.8	200.0	38.6	37.8	130.1	125.8	144.4	255.0	207.0	156.5
	E	90.1	477.8	55.9	139.9	258.8	376.2	336.0	715.8	514.1	575.7
Number of major leaves	A	25.0	33.2	26.6	28.1	26.4	36.2	36.7	-	-	-
	B	13.7	24.6	13.4	14.4	20.1	20.5	21.3	23.0	23.3	22.4
	C	22.5	81.3	39.1	50.3	81.6	76.1	77.8	-	-	-
	D	50.8	129.9	58.8	54.9	78.4	84.8	88.8	138.4	99.7	75.0
	E	96.6	331.5	68.8	184.8	199.6	232.0	216.3	456.2	302.8	366.7
Number of fruits	A	11.0	11.8	20.2	27.2	12.1	20.9	19.8	-	-	-
	B	7.4	17.3	13.7	16.5	26.1	20.9	26.4	18.6	14.8	17.3
	C	13.4	52.4	39.4	51.8	74.5	74.5	75.3	-	-	-
	D	84.5	423.4	170.3	137.1	260.0	275.8	352.9	416.5	207.9	143.0
	E	281.7	1194.6	269.2	1044.3	746.4	1589.4	1365.1	1706.2	693.2	825.0

		Xanthium avanillesii	*Xanthium italicum*	*Xanthium pennsylvanicum*		*Xanthium chinense*			*Xanthium spinosum*		
Population numbers		44	46	25	26	11	22	42	23	27	43
Plant weight (g)	A	8.1	5.8	9.6	11.2	4.8	7.7	7.7	-	-	-
	B	7.1	11.1	7.8	8.7	12.3	10.2	12.8	2.7	2.0	2.3
	C	17.4	36.6	27.7	35.2	47.8	48.9	47.4	-	-	-
	D	64.7	290.6	100.9	69.1	113.9	122.5	199.3	69.1	33.7	22.6
	E	253.9	696.1	136.6	584.3	390.1	712.2	642.3	370.4	138.4	213.6
Days to flowering	A	67.6	69.4	65.5	65.1	69.1	64.6	67.3	-	-	-
	B	94.9	118.1	69.5	67.7	116.2	114.6	116.1	99.7	101.2	102.2
	C	87.2	103.1	43.1	39.2	109.7	106.4	106.7	-	-	-
	D	80.4	108.1	66.4	66.6	113.2	108.9	111.1	86.7	89.6	89.7
	E	80.0	107.7	69.7	68.5	111.0	107.4	109.6	81.6	85.4	82.3
Mean fruit length (mm)	A	21.9	20.0	19.9	20.0	19.7	20.4	20.5	-	-	-
	B	23.8	21.5	20.8	21.2	20.4	19.9	19.6	11.2	11.5	11.1
	C	23.4	19.9	20.1	20.1	19.0	19.4	18.8	-	-	-
	D	21.4	19.5	22.9	22.6	24.8	16.6	16.4	12.3	12.4	11.6
	E	22.5	20.2	21.8	22.0	16.9	17.9	16.6	12.5	12.3	12.3
Mean fruit weight (mg)	A	346.9	152.8	139.9	186.1	140.9	173.1	154.5	-	-	-
	B	496.3	246.1	279.6	294.6	187.8	196.3	181.4	63.1	57.1	52.3
	C	521.6	192.5	274.5	255.8	159.3	209.2	182.8	-	-	-
	D	488.0	217.7	426.3	391.3	123.3	110.8	118.5	85.5	80.8	74.2
	E	466.7	198.3	367.6	342.7	118.9	142.5	104.3	88.6	85.0	88.3
Time to anthesis (days)	A	77.0	77.5	75.3	73.6	79.1	74.2	74.9	-	-	-
	B	113.8	132.4	86.1	85.3	130.1	127.9	130.8	115.8	123.2	118.9
	C	101.9	117.2	56.6	52.8	123.4	119.4	121.3	-	-	-
	D	96.6	131.9	82.8	82.4	136.1	133.7	135.1	102.7	107.3	109.2
	E	94.3	135.7	83.9	81.8	136.3	133.9	137.1	106.5	108.3	98.0

		Xanthium cavanillesii	*Xanthium italicum*	*Xanthium pennsylvanicum*		*Xanthium chinense*			*Xanthium spinosum*		
Population number		44	46	25	26	11	22	42	23	27	43
Internode distance (mm)	A	37.7	41.3	53.1	58.4	25.5	28.2	33.6	-	-	-
	B	48.5	44.1	56.8	61.4	65.7	53.1	67.9	25.1	24.3	26.1
	C	89.3	64.8	93.5	93.3	71.8	70.8	66.4	-	-	-
	D	61.6	59.9	70.8	60.6	47.7	45.2	48.4	32.2	33.5	31.9
	E	73.7	71.3	72.9	81.2	63.0	74.7	64.4	41.3	43.3	47.3

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